Executive Summary

On August 30, 1999, the South Florida Water Management District (District) contracted with DB Environmental, Inc. (DBE) to perform a 100-week evaluation of Submerged Aquatic Vegetation/Limerock (SAV/LR) Treatment System technology for reducing phosphorus (P) discharge from Everglades Agricultural Area (EAA) waters. The objectives of this project are to assess the long-term, sustainable performance of this technology, and to develop design and operational criteria for a full-scale SAV/LR system. For this effort, we are performing scientific and engineering work at Stormwater Treatment Area (STA)-1W at several spatial scales: outdoor microcosms and mesocosms, test cells (0.2 ha), Cell 4 (146 ha) and Cell 5 (1,100 ha). This document is a quarterly progress report describing work efforts of DBE's project team from February through May 2001. Key accomplishments and findings are as follows.

During this quarter, we continued using existing mesocosms and test cells at both the North and South Advanced Treatment Technology (NATT and SATT) sites of STA-1W to assess effects of hydraulic loading rates, water type (Post-BMP vs. Post-STA), and system configuration on SAV/LR phosphorus removal performance. We also performed water column sampling in Cell 4 to characterize internal P concentration gradients, continued work on a mesocosm drydown (SRP release) study, and performed internal sampling in Cell 5 to characterize the SAV colonization in this cell.

During April 2001, we completed operation of a long-term, flow-through microcosm study to assess the effects of soluble reactive P, calcium and alkalinity on SAV P removal performance. SAV performance was tested at high and low Ca (65 - 90 vs. 22 – 36 mg/L with one outlier) and alkalinity (288 - 360 vs. 44 – 88 mg $CaCO_3$/L with one outlier) concentrations, as well as high (59 – 192 µg/L) and low (2 - 47 µg/L) SRP concentrations. Eight microcosms containing *Najas guadalupensis* received the various Ca/alkalinity and SRP media at a hydraulic loading rate of 9.6 cm/day (3.5 day hydraulic retention time). SAV cultured under the high Ca/alkalinity regimes provided lower outflow TP concentrations than under the low Ca/alkalinity concentrations. For the "low SRP" microcosms, outflow TP concentrations during the five month study averaged 13 and 17 µg/L under high and low Ca/alk. conditions, respectively.

Under "high SRP" conditions, outflow TP concentrations averaged 25 μg/L (high Ca/alk.) and 68 μg/L (low Ca/alk).

We observed the greatest P mass storage within vegetation and sediments in those microcosms that received high SRP, as well as high Ca/alk levels in the feedwaters. Sediments in the high Ca/alk treatments also were markedly enriched with Ca (~ 26% Ca) relative to those that received the low Ca/alk waters (~ 7 – 14% Ca). These data demonstrate that the rates of water column P removal, and internal storage of P in SAV systems, can be strongly linked with aqueous Ca/alk levels.

During this quarter we continued monitoring of a "drydown" study to assess export of P from dessicated sediments and vegetation following drydown and reflooding. Two mesocosms, one that previously received a high hydraulic loading (53 cm/day) of Post-BMP waters, and one that received a lower hydraulic loading (11 cm/day), are being used for the study. The mesocosms were drained and sediments were allowed to dessicate for 105 days. During this period, sediments in each mesocosm consolidated to about 35 – 40% of their original depths. As a subset of this experiment, we also assessed the export of sediment P as a function of dessication time (= moisture content). The sediments collected from the "high HLR" mesocosm intermittently exhibited SRP release to the water column, with highest release rates observed for sediments collected 14 and 29 days after the onset of dessication. By contrast, sediments collected from the "low HLR" mesocosm exhibited little SRP release during dessication.

To compare relative performance of SAV and cattail-dominated wetlands, we continued monitoring of shallow mesocosms containing these respective vegetative communities. The SAV mesocosms continued to outperform cattail mesocosms operated at similar depths (0.4m) and HLRs (10 cm/day). For the quarter we observed a 57% reduction in TP concentration (mean outflow of 24 μg/L) in the SAV-dominated mesocosms, while only a 36% decrease in TP concentration (mean outflow of 51 μg/L) was observed for the cattail mesocosms.

In order to assess performance of a SAV/periphyton system under increased flow velocities, we modified our three shallow (0.09 m deep) SAV/periphyton/LR raceways at the SATTs to

receive Post-STA waters in a sequential, rather than parallel fashion. This resulted in a tripling of the length of the flow path, which enabled us to increase the HLR and flow velocity (to 0.36 cm/sec.) without changing the HRT. This higher flow velocity, however, did not result in reduced outflow P concentrations from the raceways. During the six-month study, inflow and outflow TP concentrations averaged 21 and 17 µg/L.

During this quarter we initiated our second small-scale filtration study at the SATT site. In this experiment, eight 208 L barrels filled with 30 cm of either limerock or Pro-Sil Plus (2 – 3 mm nominal diameter) are being fed post-STA waters. Total P removal performance of these "polishing" filters will be evaluated over a 13-week period.

During March 2001, our project team began evaluating the P removal performance of a Chemical Treatment/Solids Separation (CTSS) pilot treatment plant, operated in tandem with an SAV-dominated test cell (NTC-14). Most of our effort this quarter was devoted to optimizing chemical dosages and clarifier performance.

For most of the quarter, the four 0.2 ha SAV test cells were operated at HLRs of 11 cm/day (north cells) and 5 cm/day (south cells). Despite the high HLRs, the north test cells continued to exhibit good performance, providing mean outflow TP concentrations of 18 µg/L. Our prior experience with SAV mesocosms indicated that comparable TP outflow levels can be achieved with even higher HLRs. We therefore increased flows to both NTC-1 and NTC-15 at the end of the quarter to approximately 25 cm/day. In contrast with the exemplary performance of the NTCs, the south test cells provided little TP removal (range of 8 – 12%) during the quarter, equivalent to outflow TP concentrations of 21 and 22 µg/L.

We performed water quality sampling that spanned 25 internal stations within the SAV-dominated Cell 4 on two dates, February 8 and April 12, 2001. Highest water column TP levels were observed near the inflow region, and along prominent short-circuit paths that exist along western and eastern boundaries of the wetland.

In February 2001, we evaluated SAV colonization at the 120 monitoring stations that we had established in STA-1W Cell 5 approximately a year earlier. The submerged species *Najas* and *Ceratophyllum* exhibited a distribution throughout the wetland similar to that observed during the previous quarter. By contrast, the aquatic weed *Hydrilla verticillata* appeared to expand in both distribution and standing crop biomass throughout the wetland.

Table Of Contents

List of Figures

List of Tables

Introduction

On August 30, 1999, the District contracted with DB Environmental, Inc. (DBE) to design, construct, operate, and evaluate a 100-week, multi-scale demonstration of SAV/Limerock Treatment System technology for reducing phosphorus (P) discharge from Everglades Agricultural Area (EAA) waters. The objectives of this project are to:

- Design and execute a scientific and engineering research plan for further evaluation of the technical, economic and environmental feasibility of using the SAV/LR system for P removal at both the basin and sub-basin scale.

- Obtain samples adequate to conduct a Supplemental Technology Standard of Comparison (STSOC) analysis.

- Provide information and experience needed to design a full-scale SAV/LR system.

This document is a progress report for the sixth quarter describing work efforts during February – May 2001. We included 4 months (instead of 3 months) as the reporting period since this is the last progress report before the final report, and the added month allowed us time to provide complete data analyses for some subtasks from start to finish. This report focuses on methodology and findings from the mesocosm experiments (Task 5), test cell studies (Task 6), Cell 4 performance monitoring (Task 9), Cell 5 inoculation studies (Task 10), and stable isotope analysis methodology (an amended task).

Project Team Members

DB Environmental, Inc. (DBE) is being assisted in this research and demonstration effort by several groups of engineers and scientists. The engineering firm Milian, Swain and Associates from Miami, FL, is providing technical field support for this project. The scientific and engineering firm HSA/Conestoga Rovers and Associates (West Palm Beach, FL) is providing engineering design and field assistance with several filtration components of this study. Particle analyses and assessments of dissolved organic P stability are being performed by the Soil and

Water Science Department of the University of Florida, Gainesville, FL. Engineering issues pertaining to full-scale SAV wetlands are being addressed by Wetland Studies and Solutions, Inc., of Chantilly, VA.

Task 5. Mesocosm Investigations

During February - May 2001, we continued using existing mesocosms at both the North and South Advanced Treatment Technology sites of STA-1W to assess effects of hydraulic loading rates, water type (Post-BMP vs. Post-STA), and system configuration on P removal performance. Several new experiments (e.g. Filter Media, experiment 2 (Subtask 5x)) were initiated during the quarter, while others were concluded (e.g. Coprecipitation, Experiment 2 (Subtask 5i)). Activities are listed below by experimental subtask.

Effects of Calcium/Alkalinity and Soluble Reactive Phosphorus Concentrations on Phosphorus Coprecipitation (Subtask 5i)

Findings from our Phase I research using Post-BMP waters suggest that P removal in an SAV system is controlled in part by water column hardness and alkalinity. The first experiment performed in April 2000, entailed subjecting previously conditioned P-"enriched" and P-"deficient" *Najas* to a constant SRP concentration at high and low levels of calcium and alkalinity over a two-day measurement period. The relative importance of P uptake by SAV vs. coprecipitation of P in the water column was evaluated. The nutritional status of *Najas* tissues was the dominant factor influencing SRP removal rate differences between treatments.

Our second experiment began in November 2000. Factors not explored in the short-term experiment, such as the effects of SRP concentrations on the rate and extent of P coprecipitation, were pursued in this experiment, where a longer incubation period (3 months) using flow-through systems was utilized.

Methodology

Tap water from the City of West Palm Beach was selected as the experimental source water because of its consistently "low" Ca (30 mg/L) and alkalinity (56 mg $CaCO_3$/L) concentrations relative to the 71 mg Ca/L and 227 mg $CaCO_3$/L alkalinity measured in Post-BMP agricultural drainage waters (ADW). We chose to add salts to the tap water to reach the desired "high" alkalinity (375 mg $CaCO_3$/L) and Ca (100 mg Ca/L) concentrations rather than attempt to chemically remove them from the Post-BMP ADW. Opting for chemical removal would alter

the water chemistry to such a degree that it would invalidate any comparison between low Ca/alkalinity (chemically "softened") and untreated high Ca/alkalinity waters.

Because the West Palm Beach tap water has a chlorine residual and varying concentrations of SRP, dechlorination and SRP stripping were found to be necessary pre-treatment steps. This was accomplished by placing water hyacinths (*Eichhornia crassipes*) in the tap water reservoir for seven days. After the seven-day contact with water hyacinths, the water was pumped in 151L batches to four 208-liter holding reservoirs (Figure 1).

During the first month of operation, inflow amendments for each treatment were re-adjusted to represent calcium and alkalinity concentrations more typical for levels measured in post-BMP waters. Beginning November 22 the phosphorus amendment was increased from 125 to 160 µg/L. The Ca/alk amendments were reduced from target concentrations of 100 to 80 mg Ca/L and from 375 to 325 mg $CaCO_3$/L on January 26, 2001. Subsequent to these modifications, the following Ca, alkalinity, and SRP amendments were added to each of the holding reservoirs:

1. Treatment #1 – Low calcium/alkalinity (unamended) and low SRP (stripped with water hyacinth beforehand)
2. Treatment #2 – High calcium/alkalinity (amended to a final concentration of 80 mg Ca/L and 325 mg $CaCO_3$ /L) and low SRP (stripped)
3. Treatment #3 – Low calcium/alkalinity (unamended) and high SRP (stripped, then amended with 160 µg SRP/L)
4. Treatment #4 - High calcium/alkalinity (amended to a final concentration of 80 mg Ca/L and 325 mg $CaCO_3$/L) and high SRP (stripped, then amended with 160 µg SRP/L)

Although we recognize that these experimental concentrations do not represent the extreme "low" and "high" values under all environments, these treatments will henceforth be referred to as Low Ca/alk, Low SRP (treatment #1); High Ca/alk, Low SRP (treatment #2); Low Ca/alk, High SRP (treatment #3); and High Ca/alk, High SRP (treatment #4).

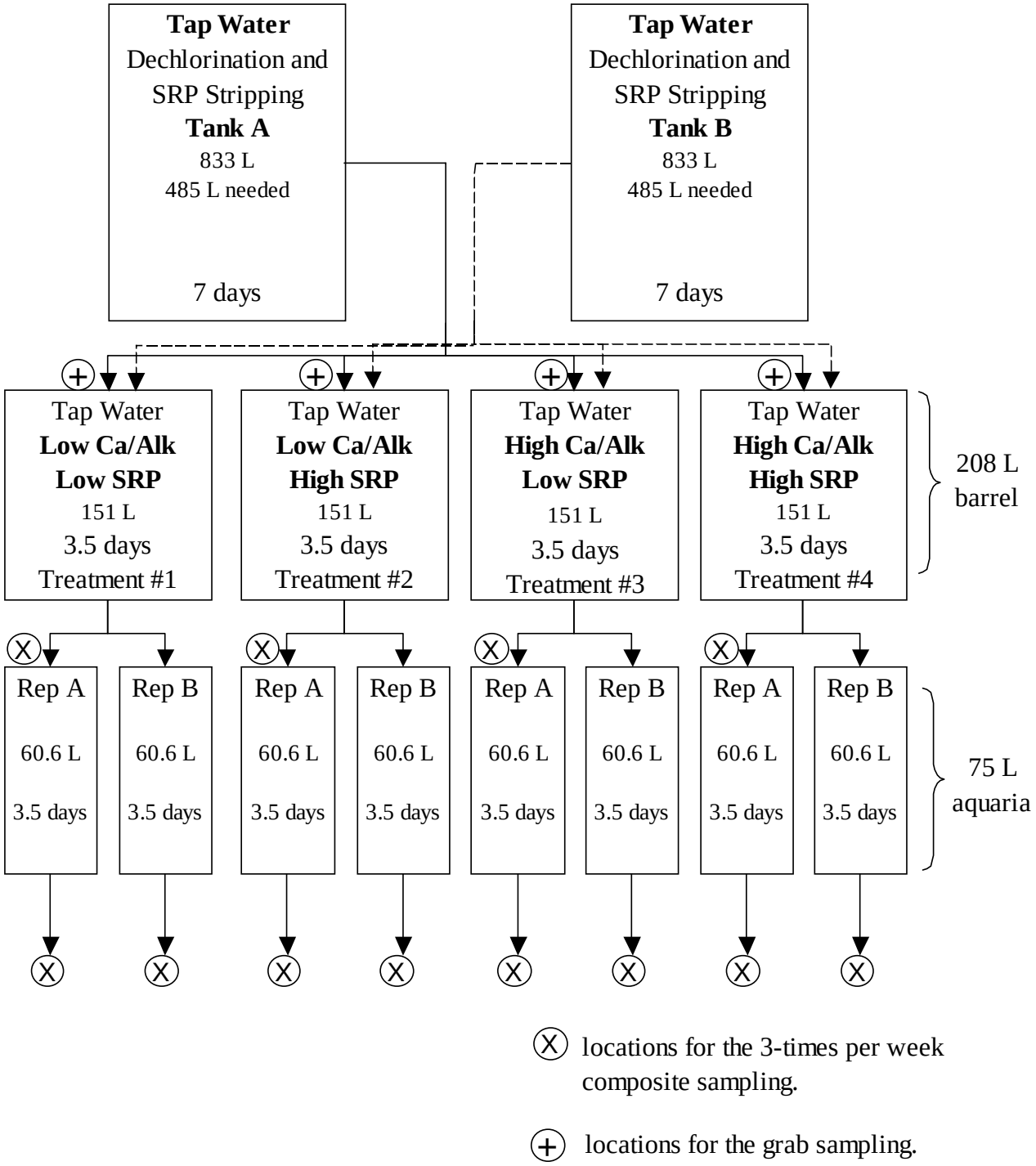

Figure 1. Flow chart detailing the experimental design for the flow-through Coprecipitation Experiment.

In addition to the Ca, alkalinity, and SRP amendments, all the barrels received inorganic N, potassium, and micronutrient supplements (Table 1). After adding the nutrient amendments, the contents of each barrel were pumped to duplicate 75-liter microcosms (Figure 1). Each of the eight microcosms (duplicates x 4 treatments) was initially stocked with 800 g (fresh wt) of *Najas guadalupensis*. No sediment was added to the microcosms.

A flow rate of 12 mL/min (17.3 L/day) was provided to each of the microcosms, which contained 60.6 L (16 gal) of water. This produced a HRT of 3.5 days (HLR=9.6 cm/day).

Composite samples of three grabs per week were collected at each sampling location (Figure 1) for TP, SRP, TSP, Ca, alkalinity, and conductance measurements. In addition to the composite sampling, we also collected grab samples after each time a new batch of source water was amended and stored within the holding tanks (Figure 1). Water temperature and pH measurements were taken in the field for each sample.

Table 1. Final concentration of nutrients and micronutrients amended to W. Palm Beach tap water.

Compound	Nutrient	Final Concentration
KCl	K^+	10.8 mg/L *
NH_4Cl	NH_4^+	0.5 mg N/L
KNO_3	NO_3^-	0.5 mg N/L
H_3BO_3	B	10.8 µg/L
$MnSO_4 \cdot H_2O$	Mn	10.8 µg/L
$ZnSO_4 \cdot 7H_2O$	Zn	5.2 µg/L
$CuSO_4 \cdot 5H_2O$	Cu	1.2 µg/L
$(NH4)_6Mo_7O_{24} \cdot 7H_2O$	Mo	0.4 µg/L
$FeCl \cdot 6H_2O$	Fe (in EDTA)	16 µg/L
Na_2EDTA	EDTA	94 µg/L
Cyanocobalimin	B-12	3 µg/L

* 9.4 mg/L from KCl and 1.4 mg/L from KNO_3

At the conclusion of five months of operation and monitoring of the coprecipitation experiment, we quantified the mass of P present in the live SAV and sediment floc within each microcosm. Along with total wet and dry weight, elemental (P, Ca, C, N) analysis of each compartment was performed for the eight microcosms.

During the five-month study, mean inflow TSP concentrations were comparable between P un-amended treatments (#1 and #2 in Table 2) and between P-amended treatments (#3 and #4). Inflow calcium and alkalinity concentrations were also comparable between like treatments, as shown in Table 2.

Table 2. Mean (± 1 s.d.) total P, TSP, SRP, dissolved Ca and alkalinity concentrations in the inflows and outflows from duplicate aquaria operated under four treatments during the five month study (November 6, 2000 – April 15, 2001).

	Treatment #1 Low Ca/alk Low P	**Treatment #2** High Ca/alk Low P	**Treatment #3** Low Ca/alk High P	**Treatment #4** High Ca/alk High P
TP (µg/L)				
Inflow	32	30	166	170
Outflow	17 ± 3	13 ± 2	68 ± 10	25 ± 2
TP Removal	15	17	98	145
TSP (µg/L)				
Inflow	22	22	157	161
Outflow	11 ± 2	7 ± 1	55 ± 9	17 ± 3
TSP Removal	11	15	102	144
SRP (µg/L)				
Inflow	12	10	136	116
Outflow	2 ± 0	2 ± 1	36 ± 9	5 ± 3
SRP Removal	15	8	100	111
Alkalinity (mg $CaCO_3$/L)				
Inflow	65	315	68	317
Outflow	69 ±3	248 ± 10	71 ± 2	230 ± 7
Alkalinity Removal	-4	67	-3	87
Diss. Calcium (mg/L)				
Inflow	27	77	28	77
Outflow	28 ± 1	50 ± 3	29 ± 1	45 ± 3
Calcium Removal	-1	28	-2	31

Phosphorus Removal and Related Water Chemistry Changes

Beginning on December 29, 2000, the outflow pH values in the High and Low SRP treatments receiving Ca and alkalinity amendments began to diverge (Figure 2). Although the outflow pH in both sets of treatments began to increase on that date, the higher pH values tended to be more associated with the High SRP treatment, which also increased the calcium carbonate saturation index (Table 3) and resulted in more precipitation. Accordingly, outflow calcium and alkalinity concentrations were slightly lower for the High than the Low SRP treatment receiving Ca/alk salts (Figure 3). A similar divergence in pH levels, corresponding to the same time period, also occurred in both sets (High and Low SRP) of the unamended Ca/alk treatments (Figure 2), though reductions of calcium and alkalinity concentrations did not occur (Figure 4).

The most likely explanation for the higher pH values associated with the effluents of the SRP-amended treatments is the change in the nutritional status of the *Najas*. Initially, the P content of the *Najas* inoculated into all the treatments was 1306 mg/kg. After 8 weeks of exposure (November 6 - December 29, 2000) to SRP concentrations of 7 ± 5 µg/L, the *Najas* became P "deficient" in the P-unamended treatments. By contrast, *Najas* in the P-amended microcosms receiving SRP concentrations of 99 µg/L (High Ca/alk) and 125 µg/L (Low Ca/alk) became P "enriched". The result of the different P treatments was that after 8 weeks of exposure, the *Najas* populations that received the higher SRP concentrations had higher rates of primary production than the populations grown in low SRP medium, regardless of whether they were grown in High or Low Ca/alkalinity water.

Along with enhanced pH elevations observed in the High Ca/alk, High SRP microcosm outflows after December 29th, concentration reductions of Ca and alkalinity after that date were greater than those observed during the initial eight weeks of operation (Figure 3). Outflow waters were more supersaturated with respect to $CaCO_3$ after December 29 than before (Table 3), due primarily to higher observed pH levels. The same trends were less pronounced in the High Ca/alk, Low SRP treatments (Figure 4) due to poorer P nutrition of the *Najas*, and subsequently smaller pH elevations, as discussed above.

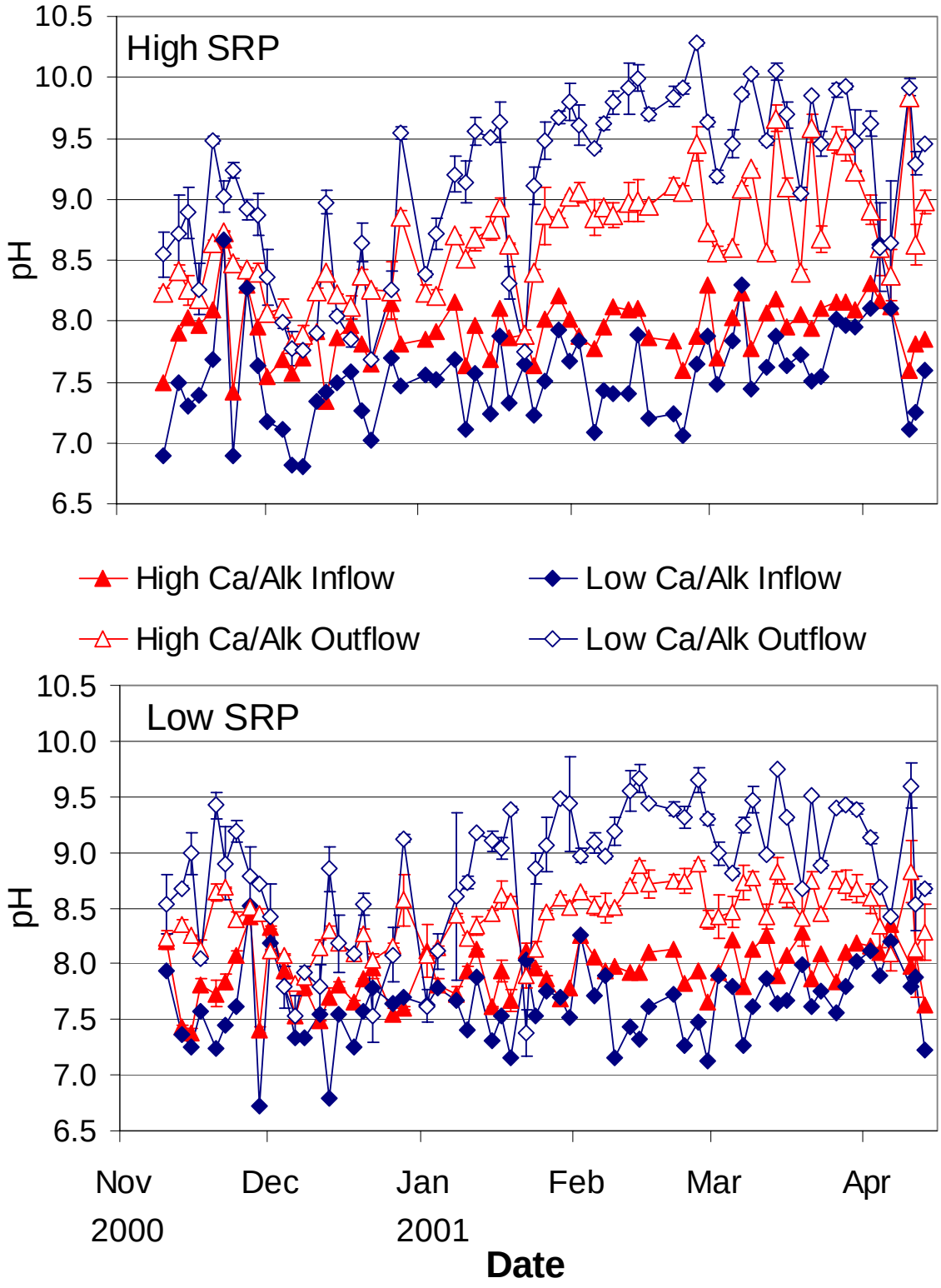

Figure 2. Mean inflow and outflow pH levels from microcosms which received tap water that was either unamended (Low) or amended (High) with concentrations of SRP and Ca/alkalinity for 160 days.

Table 3. $CaCO_3$ saturation indexes (SI) and pH values in the inflow and outflow waters from microcosms which received tap water unamended (Low) and amended (High) with SRP and Ca/alkalinity for 160 days.

	Inflow				Outflow			
	pH	s.d.	SI	s.d.	pH	s.d.	SI	s.d.
Entire Period of Record, November 6, 2000 through April 15, 2001								
Low Ca/alk, Low SRP	**7.62**	0.33	**0.3**	0.2	**8.85**	0.59	**7.3**	6.4
Low Ca/alk, High SRP	**7.54**	0.40	**0.4**	0.9	**9.14**	0.70	**15.0**	13.9
High Ca/alk, Low SRP	**7.91**	0.24	**5.7**	1.7	**8.42**	0.26	**9.2**	3.2
High Ca/alk, High SRP	**7.93**	0.24	**6.0**	1.9	**8.67**	0.45	**13.2**	5.6
Before December 29, 2000								
Low Ca/alk, Low SRP	**7.55**	0.39	**0.2**	0.1	**8.46**	0.54	**2.6**	1.8
Low Ca/alk, High SRP	**7.40**	0.45	**0.2**	0.1	**8.53**	0.56	**3.6**	2.7
High Ca/alk, Low SRP	**7.80**	0.29	**5.2**	1.6	**8.26**	0.22	**7.4**	2.1
High Ca/alk, High SRP	**7.87**	0.31	**6.1**	1.9	**8.30**	0.24	**8.7**	2.6
After December 29, 2000								
Low Ca/alk, Low SRP	**7.66**	0.28	**0.3**	0.2	**9.06**	0.51	**9.9**	6.5
Low Ca/alk, High SRP	**7.62**	0.35	**0.6**	1.1	**9.46**	0.52	**21.5**	13.4
High Ca/alk, Low SRP	**7.97**	0.19	**5.9**	1.7	**8.51**	0.24	**10.3**	3.3
High Ca/alk, High SRP	**7.97**	0.19	**6.0**	1.9	**8.87**	0.41	**15.8**	5.1

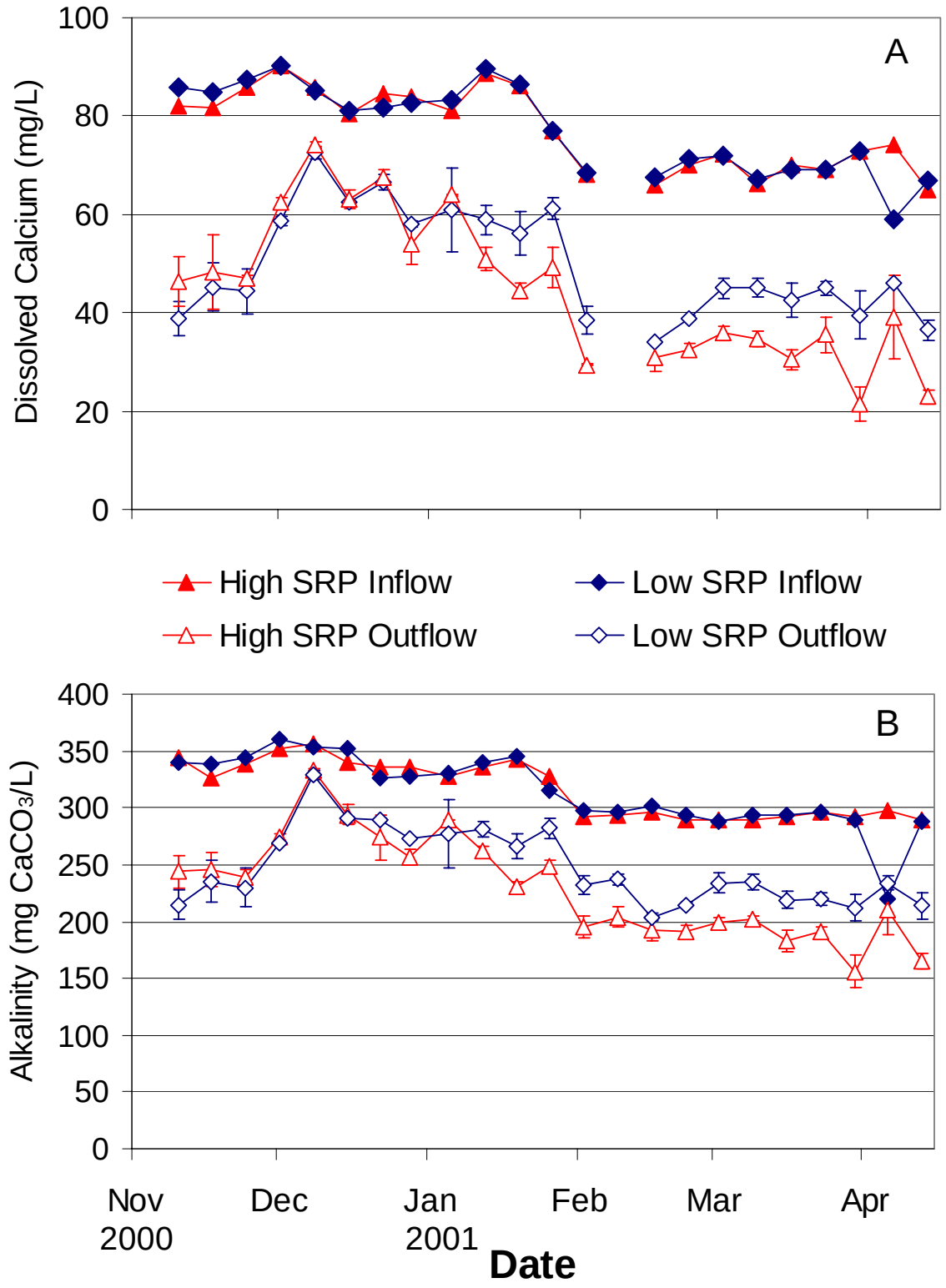

Figure 3. Mean inflow and outflow (A) dissolved calcium and (B) total alkalinity concentrations from microcosms which received tap water amended with Ca/alkalinity and either unamended (Low) or amended (High) with SRP for 160 days.

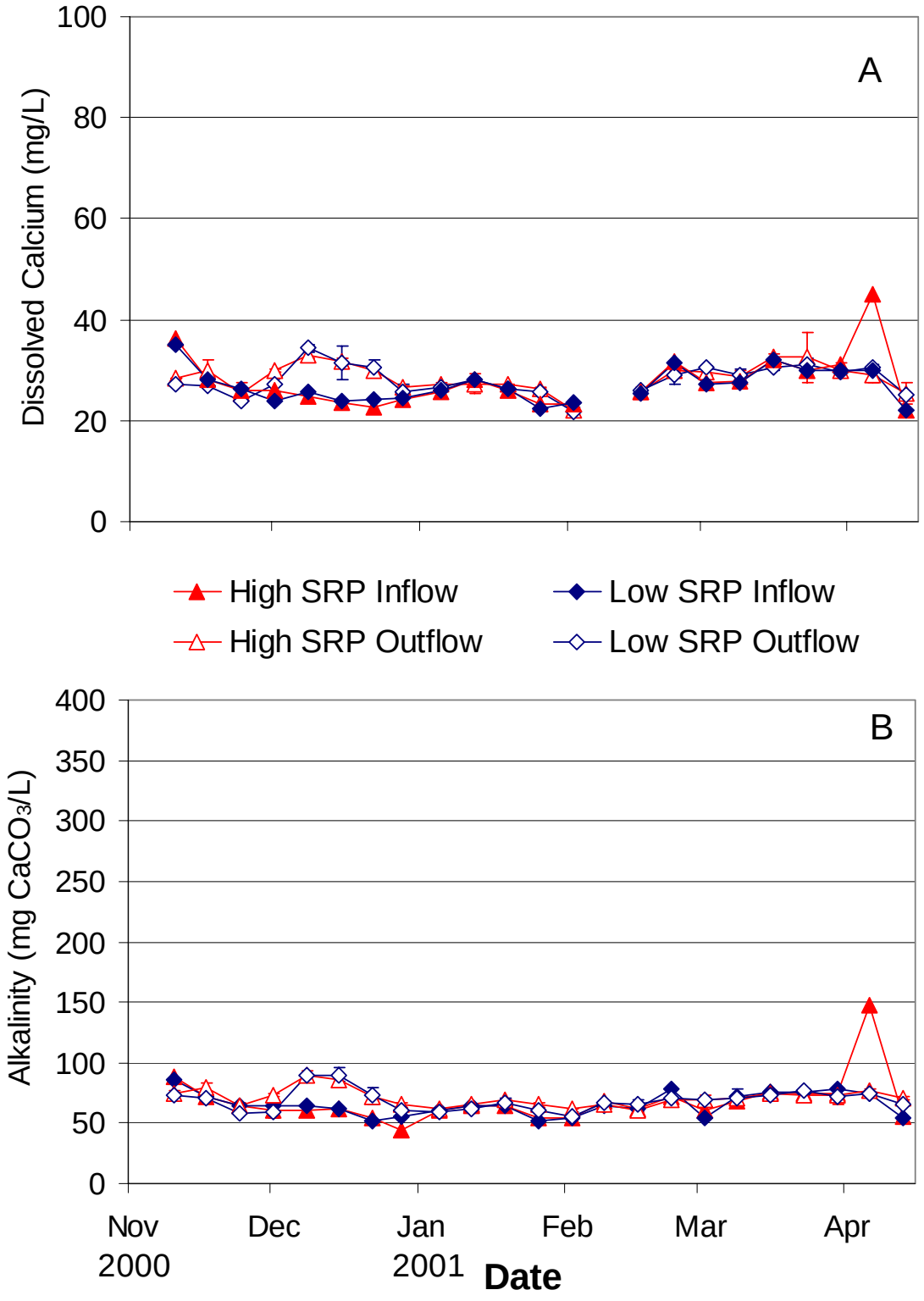

Figure 4. Mean inflow and outflow (A) dissolved calcium and (B) total alkalinity concentrations from microcosms which received tap water without Ca/alkalinity amendments, and either unamended (Low) or amended (High) with SRP for 160 days.

Among the Ca/alk-amended treatments, the inflow waters were supersaturated with respect to $CaCO_3$ in both the P-amended and unamended treatments (saturation index, SI = 6.0 and 5.7, respectively). The inflow waters to the treatments without Ca/alk additions were undersaturated (SI = 0.3-0.4), and although photosynthetically-driven pH elevations created supersaturated conditions in the outflow from those systems (Table 3), no net removal of Ca or alkalinity occurred over the five-month monitoring period (Table 2). In the Ca/alk-amended treatments, however, Ca and alkalinity (as $CaCO_3$) were removed in nearly a 1:1 molar ratio (Figure 5), indicating that precipitation of $CaCO_3$ was the primary removal mechanism in those treatments.

Considering that both SRP and dissolved organic carbon (and thus dissolved organic P [DOP]) can both be coprecipitated with $CaCO_3$ (Seuss 1970; Murphy et al. 1983; Danen-Louwerse et al. 1995), we examined the TSP component of our water quality data for the relevance of the P coprecipitation process. Within the P-amended treatments, TSP concentration was reduced to a greater extent in the High Ca/alk treatment than in the Low Ca/alk treatment (Table 2, Figure 6). Since no net removal of Ca or alkalinity occurred in the low Ca/alk systems, these data suggest that coprecipitation in the P-amended mesocosms receiving High Ca/alk inflow waters accounted for ~25% of the P removal in the mineral-amended tapwater.

In the microcosms not amended with P, TSP concentration reductions were only slightly greater in the High than the Low Ca/alk treatment (15 µg/L vs. 11 µg/L). Even though the absolute TSP concentration reductions were lower in the P-unamended microcosms than the P-amended systems, the differences in the TSP removals between the High and Low Ca/alk treatments were about the same on a percentage basis (25-30%) within each treatment group. The coprecipitation of P with $CaCO_3$ in high Ca/alk mineral-amended waters therefore appeared to increase P removal by 25-30% (Figure 6), regardless of the inflow TSP concentration.

On two occasions during the five month investigation, the suite of parameters typically sampled during peak daylight hours were sampled just prior to dawn. These "snapshot" sampling events on December 28, 2000 and March 5, 2001 revealed nighttime outflow water quality (Table 4) similar to that observed during the day (Table 3), with the exception of lower pH levels at night.

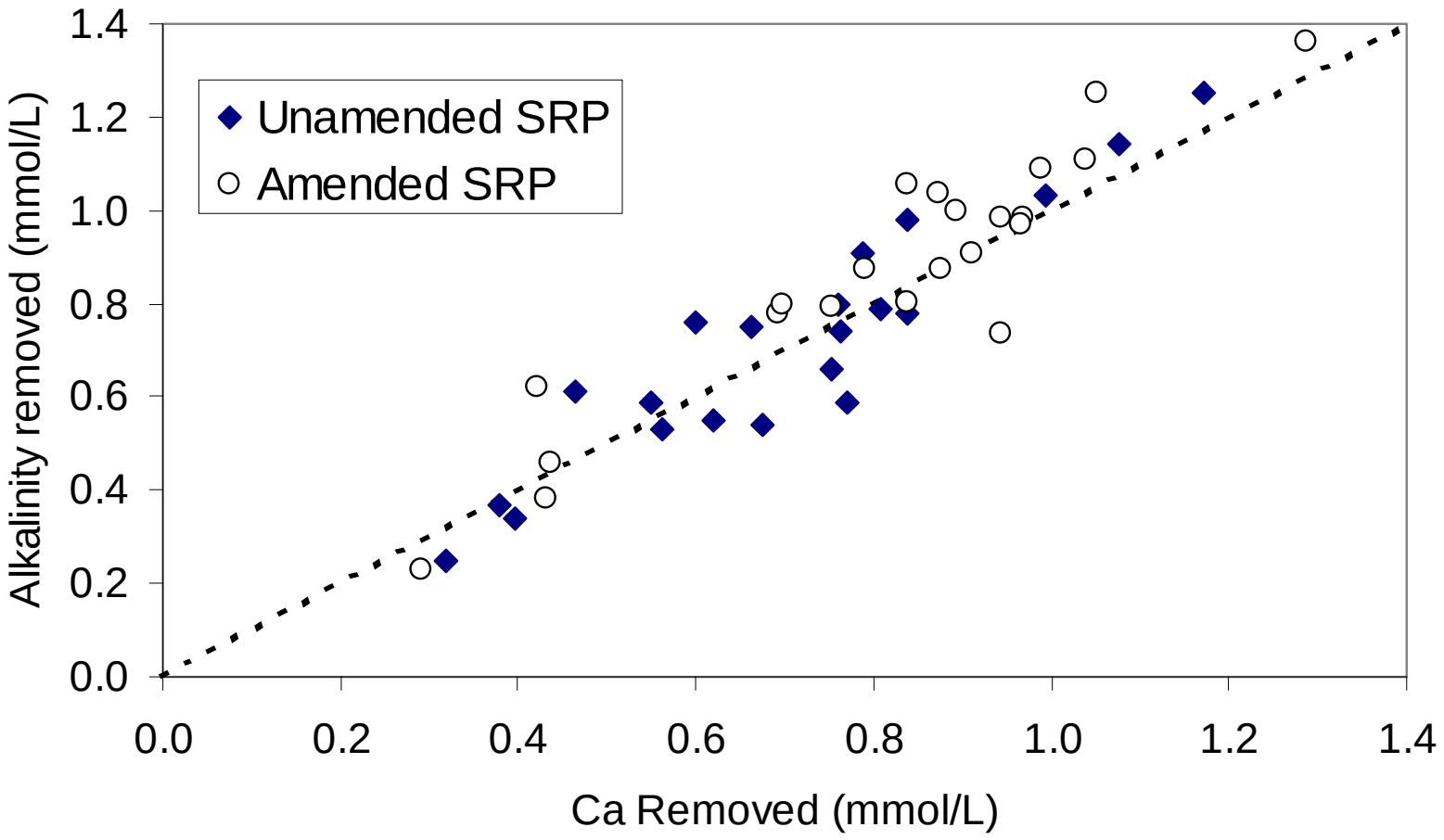

Figure 5. Calcium and alkalinity removals from amended (High) Ca/alk treatment waters with and without SRP amendments during the 160-day study. The dashed line represents removal of equal molar amounts of calcium and alkalinity as $CaCO_3$.

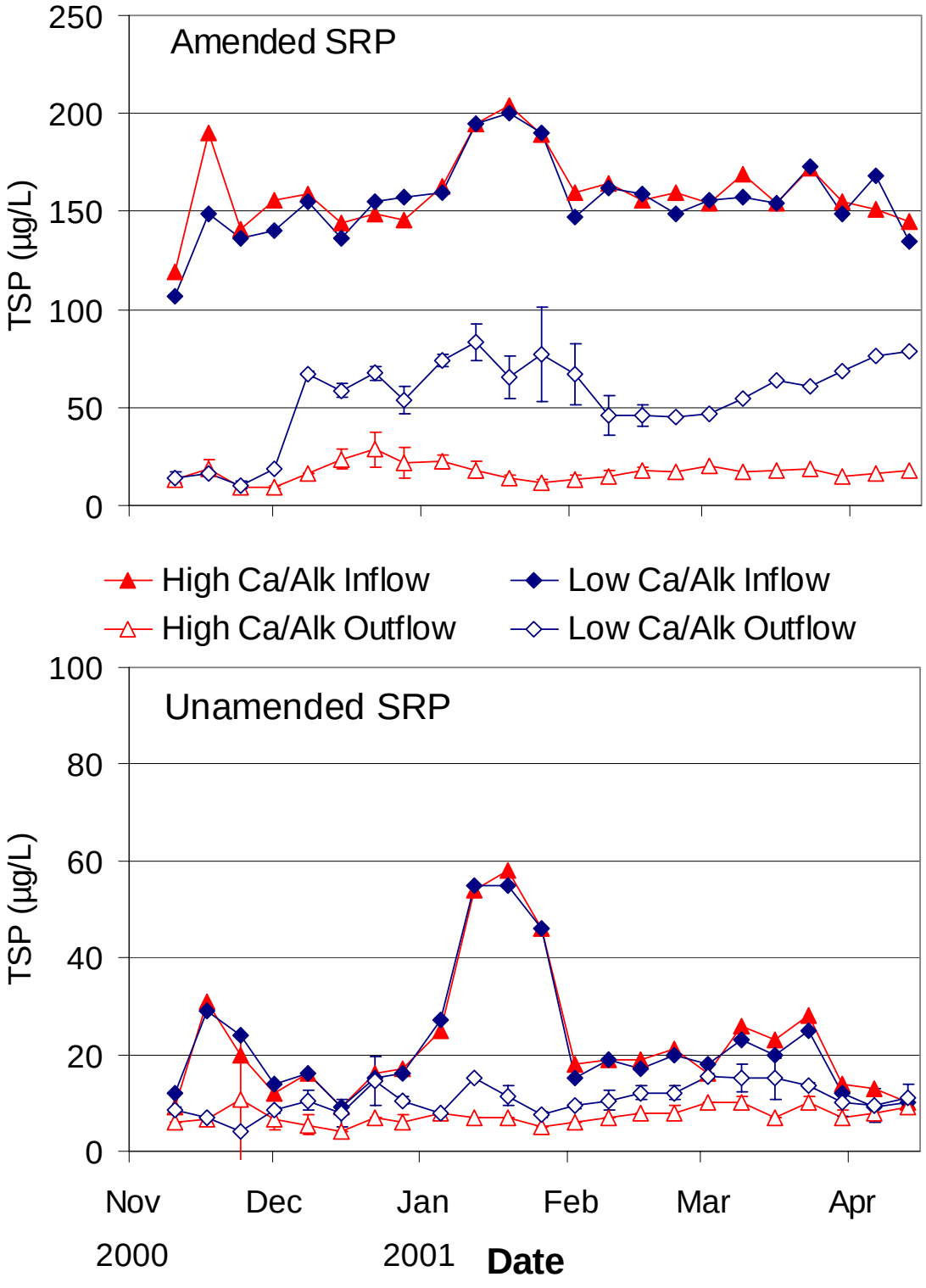

Figure 6. Mean total soluble P concentrations in the inflow and outflow waters from microcosms which received tap water unamended (Low) and amended (High) with SRP and Ca/alkalinity for 160 days.

Table 4. Concentration ranges for inflows and outflows from duplicate microcosms operated under conditions of unamended (Low) and amended (High) calcium, alkalinity, and SRP. Values represent two grab sampling events just prior to dawn on December 28, 2000 and March 5, 2001.

Parameter	Units	Low Calcium/Low Alkalinity				High Calcium/High Alkalinity			
		Inflow		Outflow		Inflow		Outflow	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
Low SRP Microcosms									
Total P	µg/L	20 - 24	*22*	13 - 24	*18*	22 - 25	*24*	10 – 16	*13*
SRP	µg/L	4 - 7	*6*	1 - 3	*2*	3 - 7	*5*	1	*1*
DOP	µg/L	9 - 12	*10*	6 - 13	*10*	9 - 18	*14*	4 – 11	*8*
PP	µg/L	4 - 8	*6*	3 - 8	*6*	4 - 6	*5*	3 – 6	*4*
Alkalinity	mg $CaCO_3$ /L	60 - 64	*62*	60 - 76	*68*	284 - 328	*306*	242 – 290	*266*
Dissolved Ca	mg/L	25 - 27	*26*	26 - 32	*29*	69 - 81	*75*	49 – 65	*57*
pH	pH	7.28 - 7.34	*7.31*	7.68 - 8.19	*7.94*	7.69 – 7.98	*7.84*	7.72 – 8.16	*7.94*
Temperature	°C	16.7 – 19.3	*18*	17.4 – 18.3	*18.8*	17.6 – 18.6	*18.1*	17.2 – 19.1	*18.2*
Specific Conductivity	µS/cm	649-788	*718*	695 – 871	*783*	1436 – 2072	*1754*	1372 – 1530	*1451*
High SRP Microcosms									
Total P	µg/L	159 – 160	*160*	44 – 67	*56*	155 – 170	*162*	17 – 27	*22*
SRP	µg/L	132 – 144	*138*	16 – 40	*28*	111 – 121	*116*	4 – 12	*8*
DOP	µg/L	11 – 18	*14*	12 – 22	*17*	28 – 48	*38*	6 – 17	*12*
PP	µg/L	5 – 9	*7*	6 – 15	*10*	6 – 11	*8*	4 – 9	*6*
Alkalinity	mg $CaCO_3$ /L	58 – 60	*59*	64 – 72	*68*	288 – 344	*316*	239 – 274	*256*
Dissolved Ca	mg/L	26 – 27	*26*	26 – 30	*28*	68 – 82	*75*	47 – 59	*53*
pH	pH	7.42 – 7.43	*7.42*	7.87 – 8.08	*7.98*	7.76 – 8.00	*7.88*	8.00 – 8.20	*8.10*
Temperature	°C	16.9 – 20.8	*18.9*	18.0 – 19.7	*18.8*	17.5 – 19.7	*18.6*	16.8 – 18.1	*17.4*
Specific Conductivity	µS/cm	638 – 793	*716*	673 – 895	*784*	1457 – 2072	*1764*	1417 – 1536	*1476*

Phosphorus and Calcium Storages

The final *Najas* standing crop biomass (526 - 785g fresh weight) in each microcosm was lower than the initial inoculum of 800g fresh weight. Despite the decline in biomass, macrophyte P mass increased over the five-month study in the P-amended microcosms due to increased tissue P concentrations (Figure 7). *Najas* tissue P concentrations declined in the P-unamended microcosms, which along with the decreased biomass, resulted in a final biomass P mass lower than the inoculum. Periphyton, where present, comprised 3-7% of the total P stored in the microcosms (Figure 8).

The total dry mass (inorganic and organic fractions) of newly accrued sediments was higher in the High Ca/alk treatments (Table 5), where $CaCO_3$ comprised 64 ± 1% of the total mass. Calcium carbonate comprised only 34 ± 2 and 18 ± 1% of the sediments recovered from the Ca/alk-unamended treatments of Low and High SRP, respectively. The sediment differences between High and Low P treatments observed in the Ca/alk-unamended (Low Ca/alk) microcosms were due to a more productive P-amended (High SRP) system depositing more organic matter in the sediments while $CaCO_3$ accrual was minimal in both systems due to low Ca/alk concentrations in the inflow waters (Table 5).

Total P concentrations in the High Ca/alk sediments were lower than in sediments receiving Low Ca/alk waters (Table 5). This was primarily due to increased $CaCO_3$ deposition in the High Ca/alk treatment systems, which reduced the relative proportion of P in those sediments. Nevertheless, on a mass and percentage basis, sediment floc-P constituted a greater fraction of the total P recovered in the High Ca/alk treatments than in the Low Ca/alk treatments having equivalent inflow P concentrations (Figures 7 and 8).

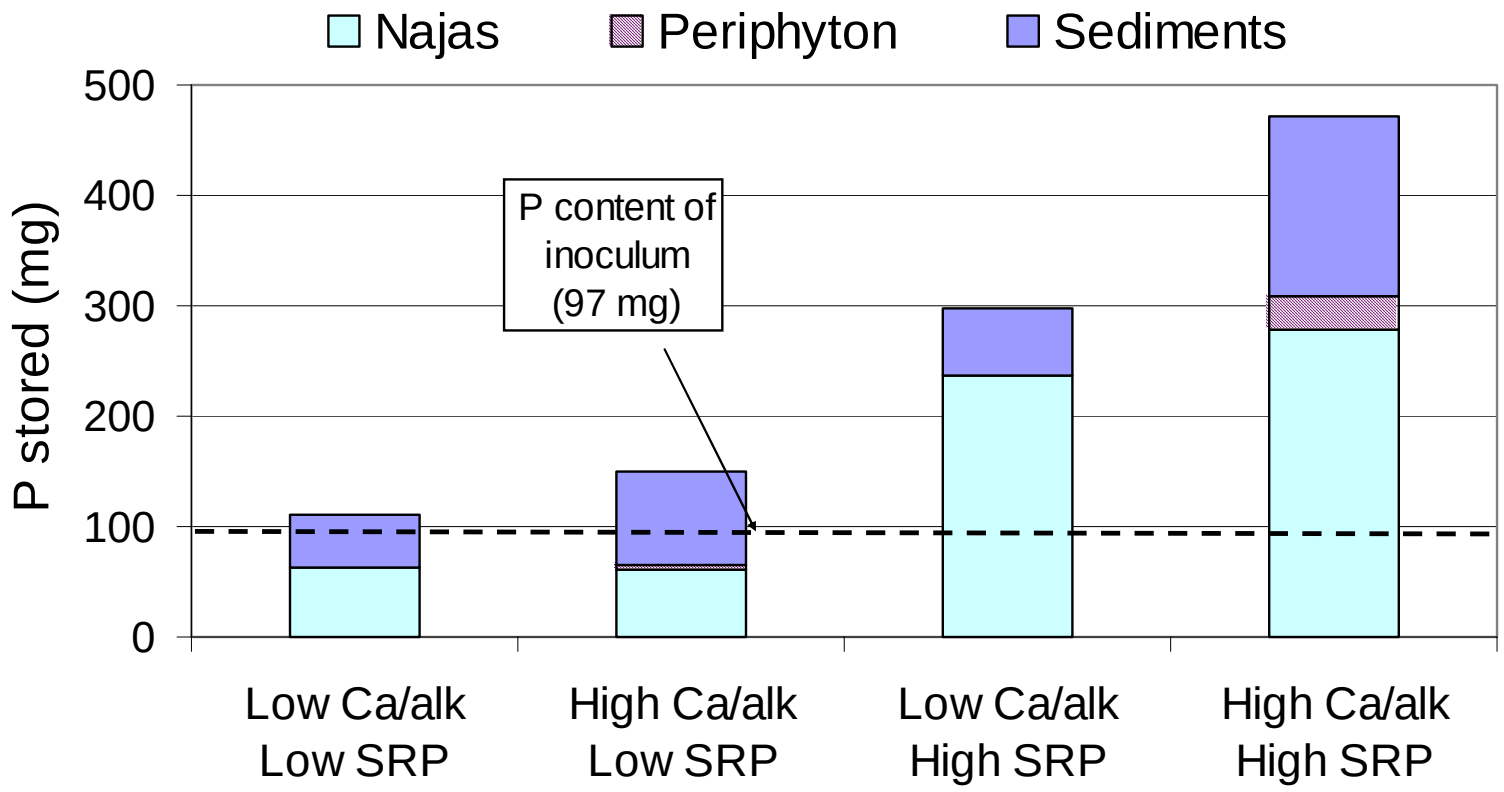

Figure 7. Phosphorus (by mass) stored in *Najas* and periphyton tissues and the sediments recovered from microcosms which received tap water unamended (Low) and amended (High) with SRP and Ca/alkalinity for 160 days. The dashed line represents the 97 mg P stored in *Najas* tissues inoculated into each microcosm at the beginning of the study.

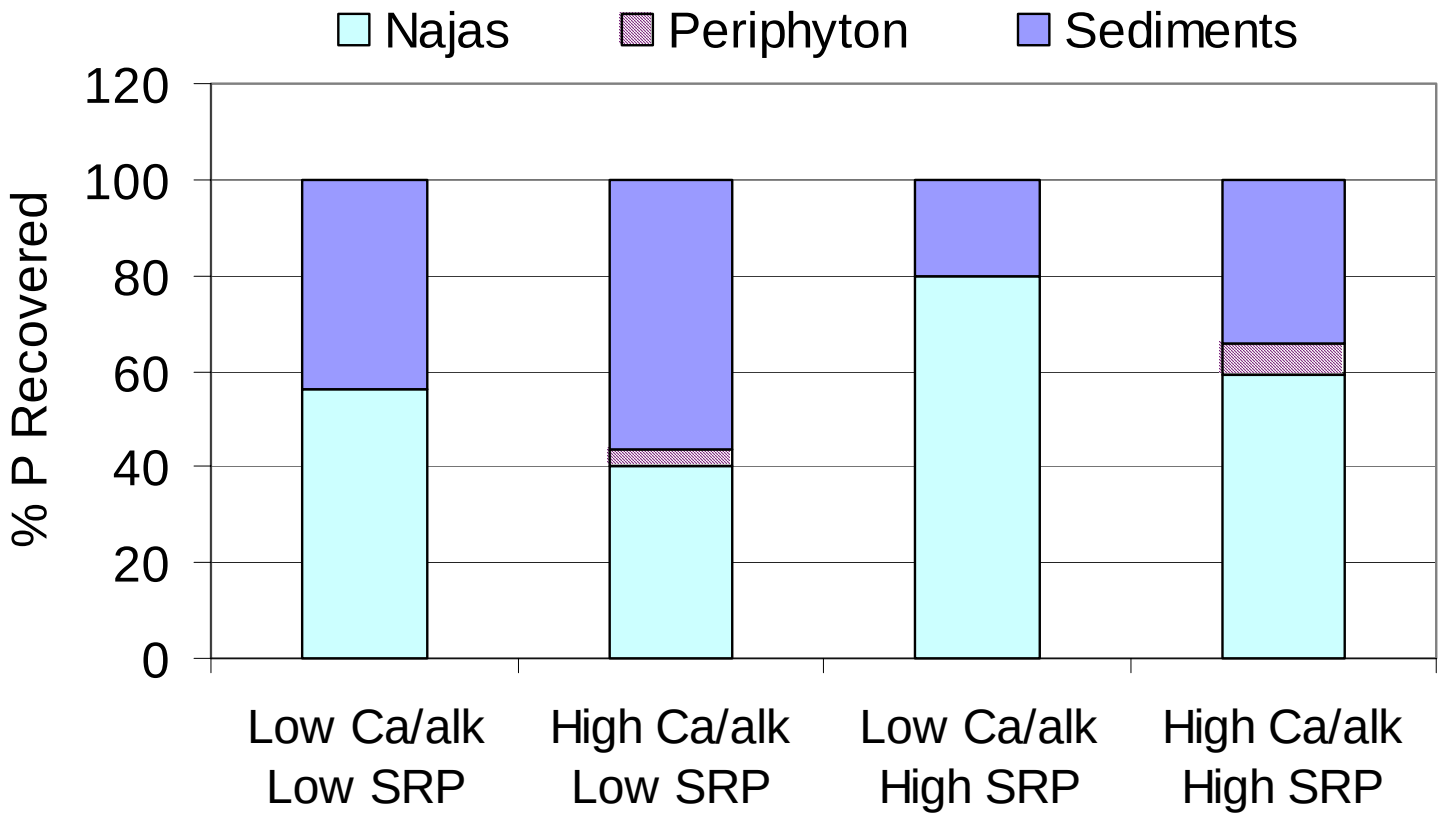

Figure 8. Distribution of phosphorus (% of P recovered) stored in *Najas* and periphyton tissues and the sediments recovered from microcosms which received tap water unamended (Low) and amended (High) with SRP and Ca/alkalinity for 160 days.

Table 5. Total deposited dry mass and total phosphorus, inorganic carbon, organic carbon and calcium concentrations of sediments collected from microcosms which received tap water unamended (Low) and amended (High) with SRP and Ca/alkalinity for 160 days.

	Total Dry Mass (g/m^2)	TP (mg/kg)	TIC (% Wt.)	TOC (% Wt.)	TCa (%Wt.)
Low Ca/alk Low SRP	199	1380	5.3	19.4	13.5
High Ca/alk Low SRP	749	643	9.0	8.2	26.0
Low Ca/alk High SRP	176	1945	5.5	22.4	7.2
High Ca/alk High SRP	795	1155	9.4	7.8	25.5

Sediment Deposition

Sediment production was influenced more by calcium and alkalinity levels than by P levels in the inflow waters (Table 5). The High Ca/alk microcosms generated more bulk sediment overall. Additionally, phosphorus storage in calcified sediments increased with higher inflow Ca/alk concentrations.

Summary

In conclusion, the findings for Experiment 2 of the P-coprecipitation subtask (5i) are in general agreement with the results reported from Experiment 1 of this subtask (DBE 2000). That is, depending on the initial SRP (and TSP), alkalinity, and hardness concentrations, and the nutritional status of the SAV, coprecipitation of soluble P compounds and ions can occur, but this process appears to account for less P removal than biological uptake by the SAV and/or bacterial communities.

Long-Term Monitoring of Phosphorus Removal Performance by SAV Mesocosms (Subtask 5iv)

After three years (start date was June 1998), monitoring of the three mesocosms operated at hydraulic retention times (HRT) of 1.5, 3.5 and 7.0 days was terminated on May 22, 2001. For this report, we will focus on the long- and short-term P removal aspects of each mesocosm. We

will limit our discussion of the plant and sediment storages to the field sampling results; treatment implications and mass balances will be presented in the final report.

The phosphorus stored within each mesocosm was quantified through vegetation harvest and sediment coring using the following methodology. Flows to each mesocosm were curtailed and the water level was reduced to a depth of 10 cm prior to plant or sediment sampling, which was performed in the "inflow" and the "outflow" region of each mesocosm. All SAV was removed from each region, separated according to species, and wet biomass weights were measured in the field immediately after harvesting. A subsample of each SAV species was returned to the lab for elemental analyses (TP, TN, TCa, TOC, TIC).

Triplicate cores were retrieved from the inflow and outflow regions of each mesocosm. After measuring sediment accretion, the accrued sediments were separated from the underlying muck (former agricultural soils) in the field and returned to the lab for bulk density and elemental analyses.

Inflow and outflow TP concentrations during the final four months of mesocosm operation (February - May 2001) were similar to the long-term average concentrations (Table 6). Limerock columns receiving mesocosm outflows continued to reduce total P concentrations, while also converting particulate P (PP) to SRP (Figure 9).

Despite periods of lower than average inflow TP concentrations (such as November 2000 – February 2001 and February-May 2001) during the three-year study, TP concentrations in the LR outflows from the 3.5- and 7.0-day HRT systems were consistently similar to average concentrations for the entire period of record (Table 6). This was due, in part, to recalcitrant DOP compounds either passing through the mesocosms or being generated within the SAV beds (Figure 9).

Table 6. Total phosphorus concentrations (µg/L) in the inflows, and SAV and limerock (LR) outflows from mesocosms operated at hydraulic retention times (HRT) of 1.5, 3.5 and 7.0 days since June 1998.

		1.5-Day HRT Outflow		3.5-Day HRT Outflow		7.0-Day HRT Outflow	
Period of Record (POR)	**Inflow**	**SAV**	**LR**	**SAV**	**LR**	**SAV**	**LR**
Entire POR June 1998 – May 2001	97	51	40	31	21	25	18
Low Inflow TP November 2000 – February 2001	42	40	28	20	17	22	18
6th (Final) Quarter February – May 2001	70	52	39	27	20	25	20

Effects of Dryout and Reflooding on Phosphorus Retention (Subtask 5v)

Upon completion of the Pulse Loading study (November 24, 2000), we began a dryout-reflooding investigation in two of the six pulsed mesocosms (one each of the high and low flow treatments) in order to assess the impact of sediment desiccation on P removal/export, to gauge the influence of previous P loading regimes on sediment stability, and to evaluate the ability of the SAV communities to recover after a dryout event.

Baseline Monitoring

This effort began with "baseline" water sampling at the inflow and outflows of the SAV and LR unit processes. During six weeks of baseline sampling the two mesocosms received Post-BMP waters at constant HLRs of 11 and 53 cm/day. Inflow and outflow TP, pH and temperature measurements were performed weekly. Total soluble P (TSP), soluble reactive P (SRP), dissolved calcium and total alkalinity were measured on biweekly composites of weekly grabs.

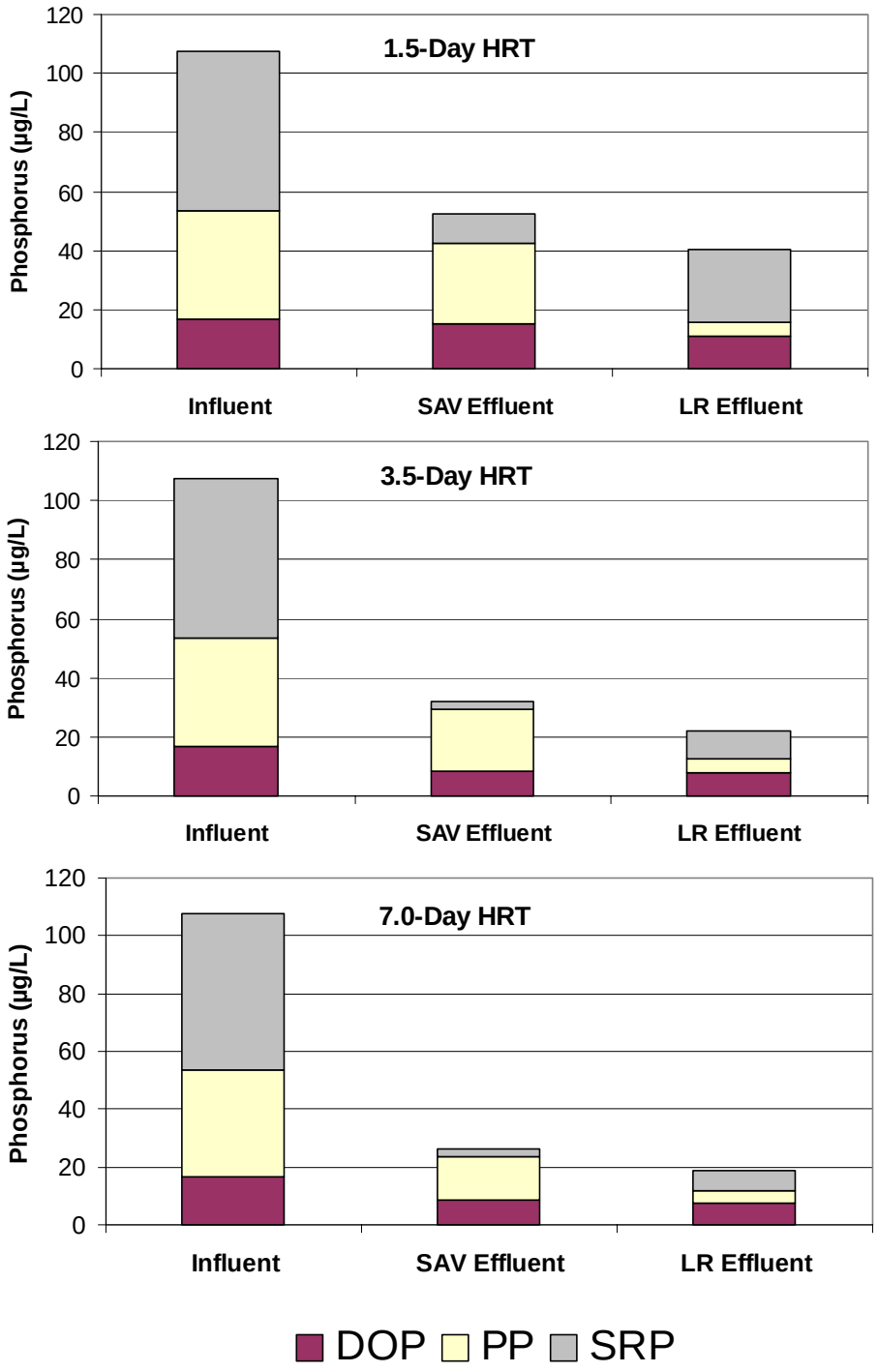

Figure 9. Dissolved organic, particulate, and soluble reactive phosphorus concentrations in the inflow and SAV and limerock (LR) outflows from mesocosms that have received Post-BMP waters at hydraulic retention times of 1.5, 3.5, and 7 days. Values represent means of weekly measurements sampled during the quarter (February-May 2001).

TP concentrations were reduced more within the lower loaded mesocosms (L-3) than the higher loaded mesocosms (S-3) (Table 7). For both treatments, slight increases in SRP concentration occurred within the LR beds. The LR bed was particularly effective in removing the PP leaving the high HLR (53 cm/day) mesocosm. DOP concentration remained unchanged through the "high-flow" mesocosm, whereas we observed a 50% reduction in DOP in the lower loaded mesocosm (Table 7).

Table 7. Chemical characteristics of the inflows and outflows of mesocosms receiving high (S-3: 53 cm/day) and low (L-3: 11 cm/day) hydraulic loading rates (HLRs), and of outflows from downstream limerock barrels, during a six-week baseline monitoring period prior to drawdown. Values represent means of six weekly grabs (TP, SRP, pH) or three two-week composites (DOP, PP, alkalinity, dissolved calcium).

	Inflow		SAV Outflow		LR Outflow	
	High HLR (S-3)	**Low HLR (L-3)**	**High HLR (S-3)**	**Low HLR (L-3)**	**High HLR (S-3)**	**Low HLR (L-3)**
TP (µg/L)	50	51	44	15	31	17
SRP (µg/L)	28	28	17	2	19	4
DOP (µg/L)	11	11	10	5	9	5
PP (µg/L)	10	12	17	8	2	8
Alk (mg $CaCO_3$/L)	197	196	193	135	193	161
Diss. Ca (mg/L)	63	64	64	41	65	49
pH	7.68	7.83	8.05	8.98	7.80	8.01

Dryout

After the six-week baseline monitoring, the water level in both mesocosms was lowered from 0.8 to 0.3 m. Small (0.30 m^2) quadrats were sampled in the inflow and outflow regions of each mesocosm to characterize the vegetation standing crop (wet and dry) and to quantify the tissue N, P and Ca contents. Vegetation was then removed from the center cross-section of each tank to facilitate sediment core retrieval, and the water level was further reduced to 1 cm to initiate the dryout period.

Characteristics of Harvested SAV

Plant decomposition, which releases biomass-P, can be a potential source for P release upon reflooding. For example, one month into the drydown period, all vegetation had decomposed in the mesocosm that had received a HLR of 53 cm/day (S-3), while in the mesocosm hydraulically loaded at 11 cm/day (L-3) much of the dried *Chara* remained intact. Therefore, the high Ca content of *Chara* tissues (Figure 10), compared to *Ceratophyllum* tissues in S-3, may attenuate P release from L-3 when the mesocosm is reflooded by retaining more P in the decomposed *Chara* biomass and by providing P sorption sites.

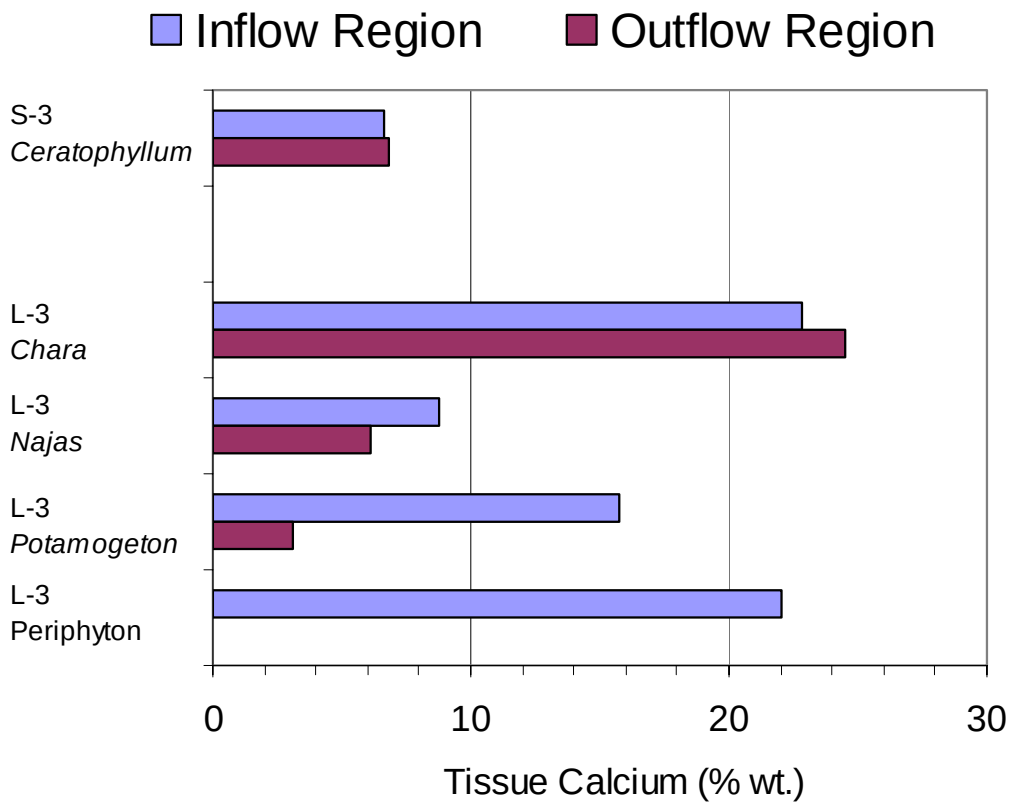

Figure 10. Calcium concentration in the tissues of dominant plant taxa in the inflow and outflow regions of mesocosms that were operated at high (S-3) and low (L-3) hydraulic loading rates of 53 and 11 cm/day, respectively.

Analysis of the initial vegetation harvested from the two mesocosms revealed differences with respect to SAV species and P content (Figure 11 and Figure 12). The S-3 mesocosm that historically received an average hydraulic loading rate (HLR) of 53 cm/day was colonized exclusively by *Ceratophyllum* whereas the lower loaded (L-3) mesocosm (HLR = 11 cm/day) supported a more diverse SAV community consisting of *Chara*, *Najas* and *Potamogeton*, as well as filamentous green periphyton, at the time of harvest (Figure 11). Higher tissue-P content was observed in the vegetation collected from the inflow than outflow regions within each mesocosm (Figure 12). This trend reflects the decreasing water column P concentrations from inflow to outflow. Despite higher P concentrations in *Ceratophyllum* tissues from S-3 (Figure 12), a similar quantity of P (by mass) had accumulated in the L-3 SAV community due to a larger standing crop biomass at the time of harvest (Figure 13).

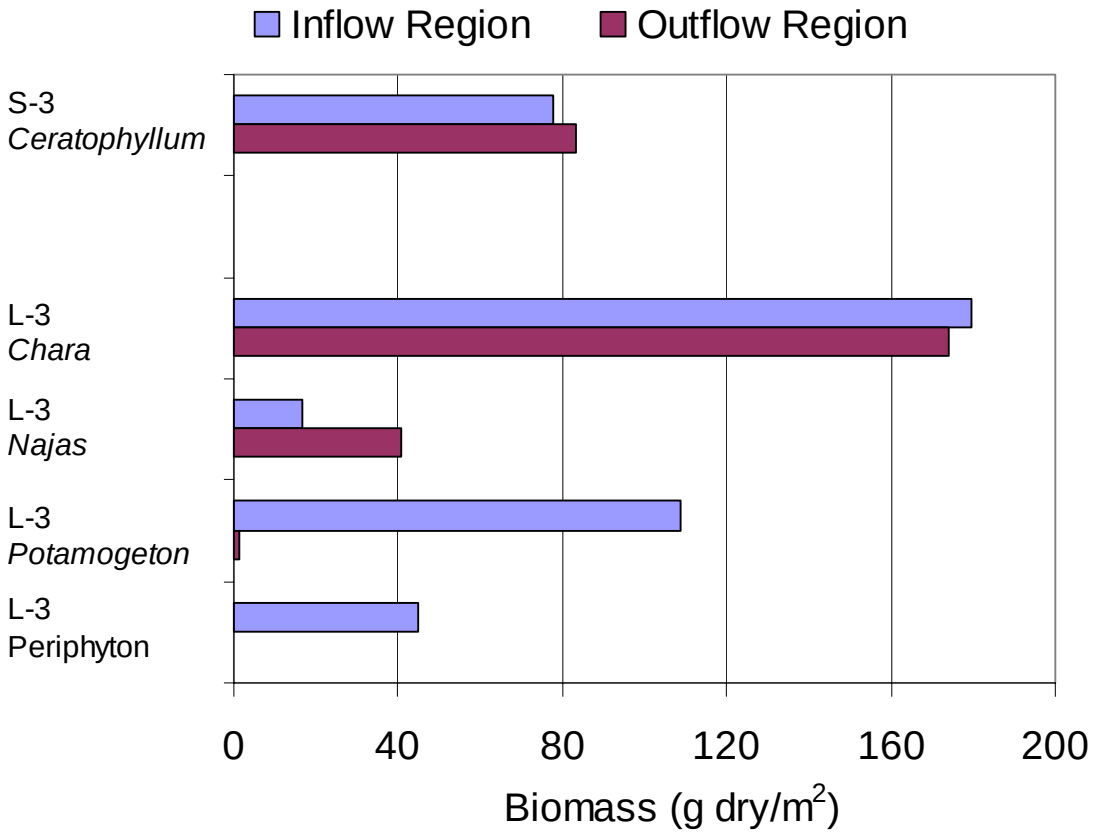

Figure 11. Standing crop biomass of dominant plant taxa in the inflow and outflow regions of mesocosms that were operated at high (S-3) and low (L-3) hydraulic loading rates of 53 and 11 cm/day, respectively.

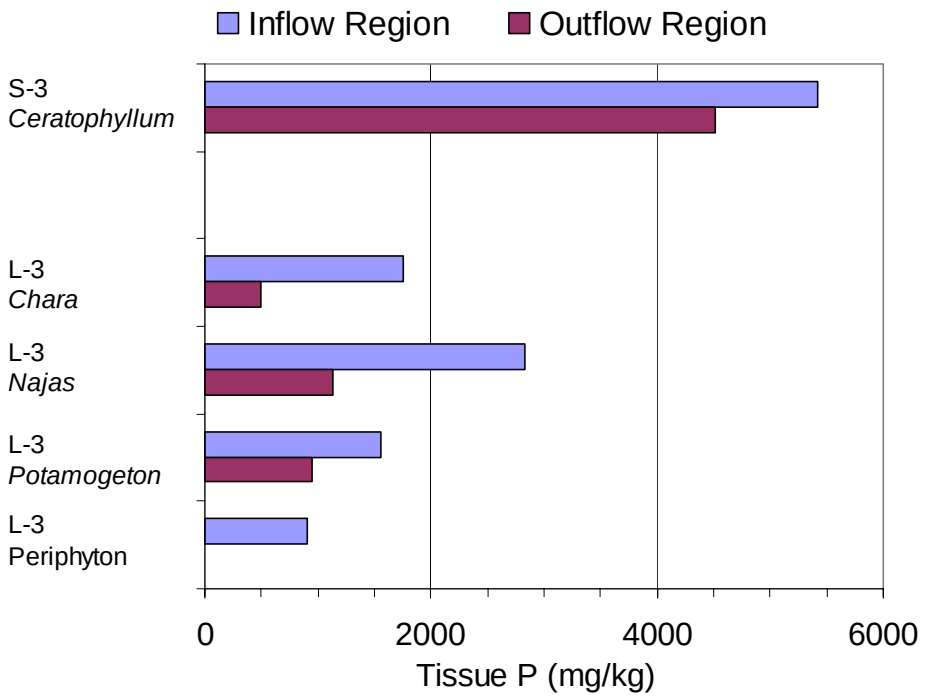

Figure 12. Phosphorus concentration in the tissues of dominant plant taxa in the inflow and outflow regions of mesocosms that were operated at high (S-3) and low (L-3) hydraulic loading rates of 53 and 11 cm/day, respectively.

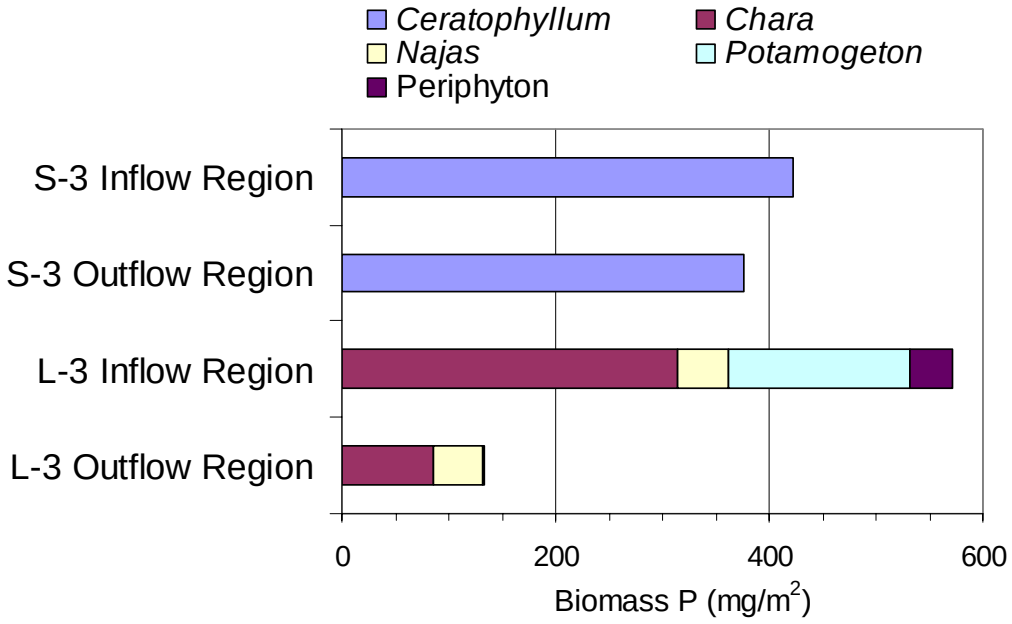

Figure 13. Phosphorus storage in the standing crops of dominant plant taxa in the inflow and outflow regions of mesocosms that were operated at high (S-3) and low (L-3) hydraulic loading rates of 53 and 11 cm/day, respectively.

Sediment Consolidation

During the desiccation period, the thickness of the newly-accrued sediment layer in each mesocosm was measured several times per week on either side of the denuded central region to record consolidation of the drying sediments. Figure 14 shows greater consolidation of sediments in the S-3 mesocosm, mostly due to the oxidation and shrinkage (dehydration) of the higher organic matter sediment in that mesocosm. After 58 days of desiccation the accrued sediment in both mesocosms had consolidated to 35-40% of the original sediment depth.

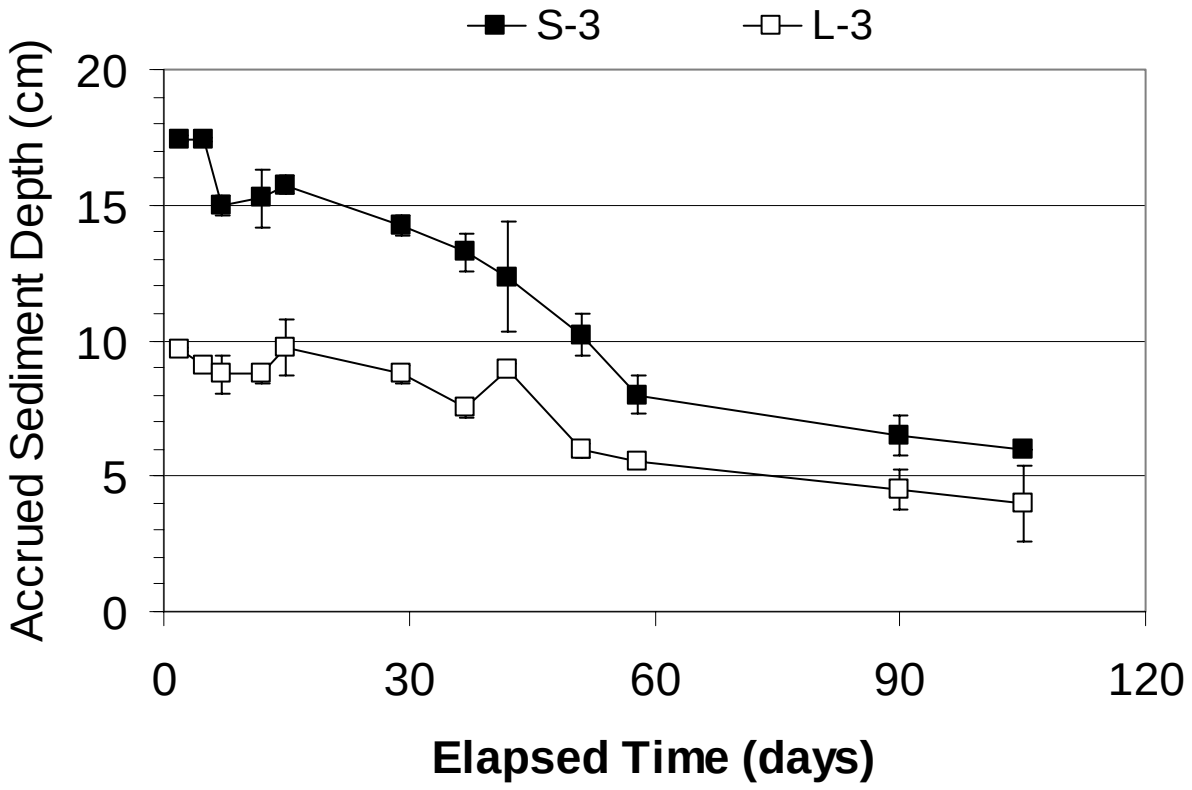

Figure 14. Consolidation during drydown of desiccating sediments originally formed under high (S-3) and low (L-3) mean hydraulic loading rates of 53 and 11 cm/day, respectively. Error bars = ± 1s.d. of duplicate measurements.

Sediment Phosphorus Concentration and Release upon Reflooding

Triplicate sediment cores (15.9 cm^2 each) were collected from the exposed central region after 0, 7, 14, 29, 58 and 105 days had elapsed since exposing the sediments to air. The accrued sediment layer, which was easily distinguished from the underlying muck, was extruded from one of the three cores and immediately dried for moisture content and P analysis. The other two intact cores retrieved from each mesocosm were rehydrated with 250 mL Cell 4 inflow water (collected on January 10, 2001 and stored in the dark at 4 ^oC between trials), and laboratory-incubated in a dark water bath (26 ±1 °C) for 24 hours. The stoppered cores were initially placed on ice during transport to the lab, and then refrigerated at 4 ^oC until rehydration and incubation which usually occurred within 48 hours of core retrieval.

We chose Cell 4 inflow water as the source water for rehydrating the cores because we believe it would represent an "average" reflood water with respect to Ca, alkalinity and P levels. Cell 4 inflow water was also the best selection since it is the water that would reflood SAV-dominated Cell 4 should it ever dry out. Initial characteristics of the overlying water are provided in Table 8. A control (identical acrylic core with 250 mL overlying water and without sediment) was also incubated with the four sediment cores during each trial.

Table 8. Characteristics of sediment core incubation water collected from Cell 4 Inflow on January 10, 2001.

	pH	TP	SRP	DOP	PP	Alkalinity	Ca
Cell 4 Inflow	7.81	36 µg/L	14 µg/L	13 µg/L	9 µg/L	302 mg/L	82 mg/L

Phosphorus concentrations in the two sediment types (S-3 and L-3) remained similar over the five sampling events to those measured prior to drydown, exhibiting an average of 1330 ±108 mg P/kg in S-3 sediments and 686 ±107 mg P/kg in L-3 sediments (Figure 15).

Incubation water pH values increased equally across all treatments during each trial. Initial pH values of 7.81 – 8.27 increased to 8.33 - 8.85 after 24 hrs. Sediment moisture content remained unchanged during the first month of the desiccation period, with 88 ±2% water found in cores

across all treatments (S-3 and L-3) and trials (ΔT = 0, 7, 14 and 29 days). Sediment moisture content decreased in both treatments to 81 ±1% at ΔT = 58 days, and after 105 days, S-3 sediments declined to as low as 53%. Sediments within both mesocosms had thoroughly dried and cracked after 105 days, causing separation from the walls of the mesocosm tanks.

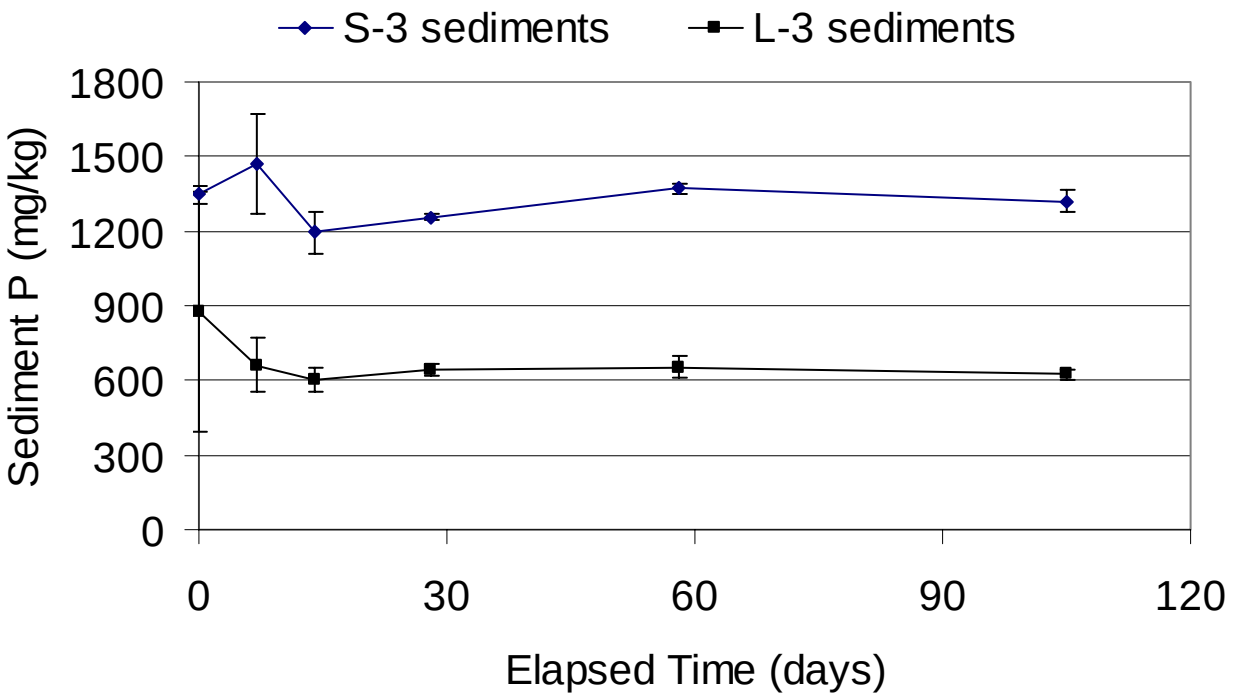

Figure 15. Phosphorus concentration of desiccating sediments from mesocosms that were operated under high (S-3) and low (L-3) hydraulic loading rates of 53 and 11 cm/day, respectively, prior to the onset of desiccation. Error bars = ± 1 s.d.of triplicate cores.

During incubation of cores collected immediately after drawdown (ΔT = 0 days), overlying water SRP concentrations were reduced from 14 µg/L to below the method detection limit (MDL) of 2 µg/L in cores retrieved from the low loading mesocosm (L-3) (Figure 16). In contrast, SRP concentrations were relatively unchanged in the high loading mesocosm (S-3) cores (11 ±4 µg/L). Control overlying water (no sediment) was reduced from 14 to 6 µg SRP/L during the 24-hour incubation.

For the ΔT = 7 day post-desiccation 24-hr core incubations, SRP concentrations were reduced from 13 to ≤ 3 µg/L in S-3, L-3 and control (no sediment) cores. At ΔT = 14 days, however, differences were again observed between treatments. SRP concentrations increased from 14 to 57 µg/L in the overlying water of the S-3 sediment, while waters above L-3 sediments and in the control core were reduced to 2 µg SRP/L (Figure 16). SRP release from the incubating S-3 sediments was less at ΔT = 29 days, and by ΔT = 58 days, the overlying water SRP concentration was reduced over the 24 hour incubation period in all treatments. The final incubation of S-3 and L-3 sediments took place after 105 days of desiccation. Sediments from both treatments were noticeably drier than during previous iterations, yet no SRP release was observed during the 24-hour experimental rehydration.

Phosphorus release from drying sediments was expected *a priori* to be greater from S-3 sediments than from L-3 sediments, due to higher TP in the former (DBE 1999). Sediments formed under higher nutrient loadings (such as S-3 and Cell 4 Inflow) typically exhibit higher TP and organic matter concentrations and lower Ca concentrations. Consequently, they release more P upon reflooding when compared to sediments formed under lower nutrient loadings (e.g., L-3 and Cell 4 Outflow locations).

After the last core retrieval on April 25, 2001 (ΔT = 105 days of desiccation), the mesocosms were reflooded at the original hydraulic loading rates of 11 and 53 cm/day. Outflow TP concentrations will be monitored until July 20, 2001, when the experiment is scheduled for termination.

Comparison of Phosphorus Removal Performance by Cattail- and SAV-Dominated Systems (Subtask vi)

Superior performance by SAV-dominated systems over cattails has been demonstrated by this mesocosm-scale experiment, where one SAV- and two cattail-dominated mesocosms (water depth = 0.4 m) were hydraulically loaded at 10 cm/day. The SAV and the two cattail mesocosms have provided average outflow TP concentrations of 24, 41 and 62 µg/L, respectively, for the period December 29, 1998 to May 21, 2001 (Figure 17).

During this quarter, SAV- and cattail-dominated mesocosms reduced inflow TP concentrations by 57 and 36±8%, respectively. Particulate P and SRP concentrations were lower in the SAV outflow than in the cattail outflow, while DOP concentrations remained constant through the cattail-dominated system but decreased in the SAV mesocosm (Figure 18).

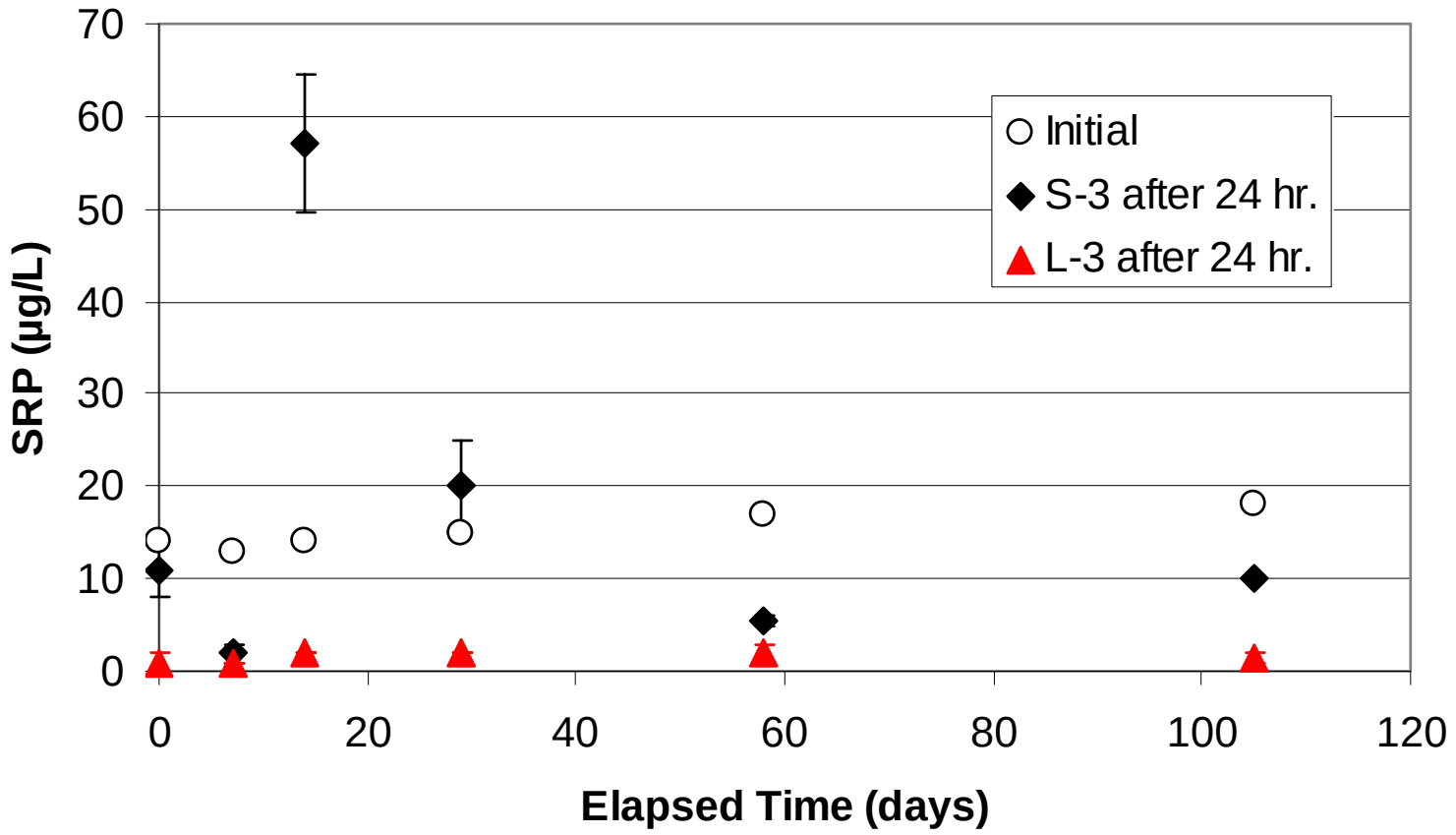

Figure 16. The change in SRP concentration in 250-mL of overlying water during 24-hour incubations of sediment cores retrieved from mesocosms that were operated under high (S-3) and low (L-3) hydraulic loading rates of 53 and 11 cm/day, respectively, prior to the onset of desiccation (elapsed time of 0 days). The initial SRP is the concentration in the overlying water prior to exposure to sediment; the S-3 and L-3 data points are the post-incubation SRP concentrations in the overlying water. The S-3 and L-3 data points represent the mean of two replicate cores (bars = ranges) after a 24 hour incubation in contact with the sediments.

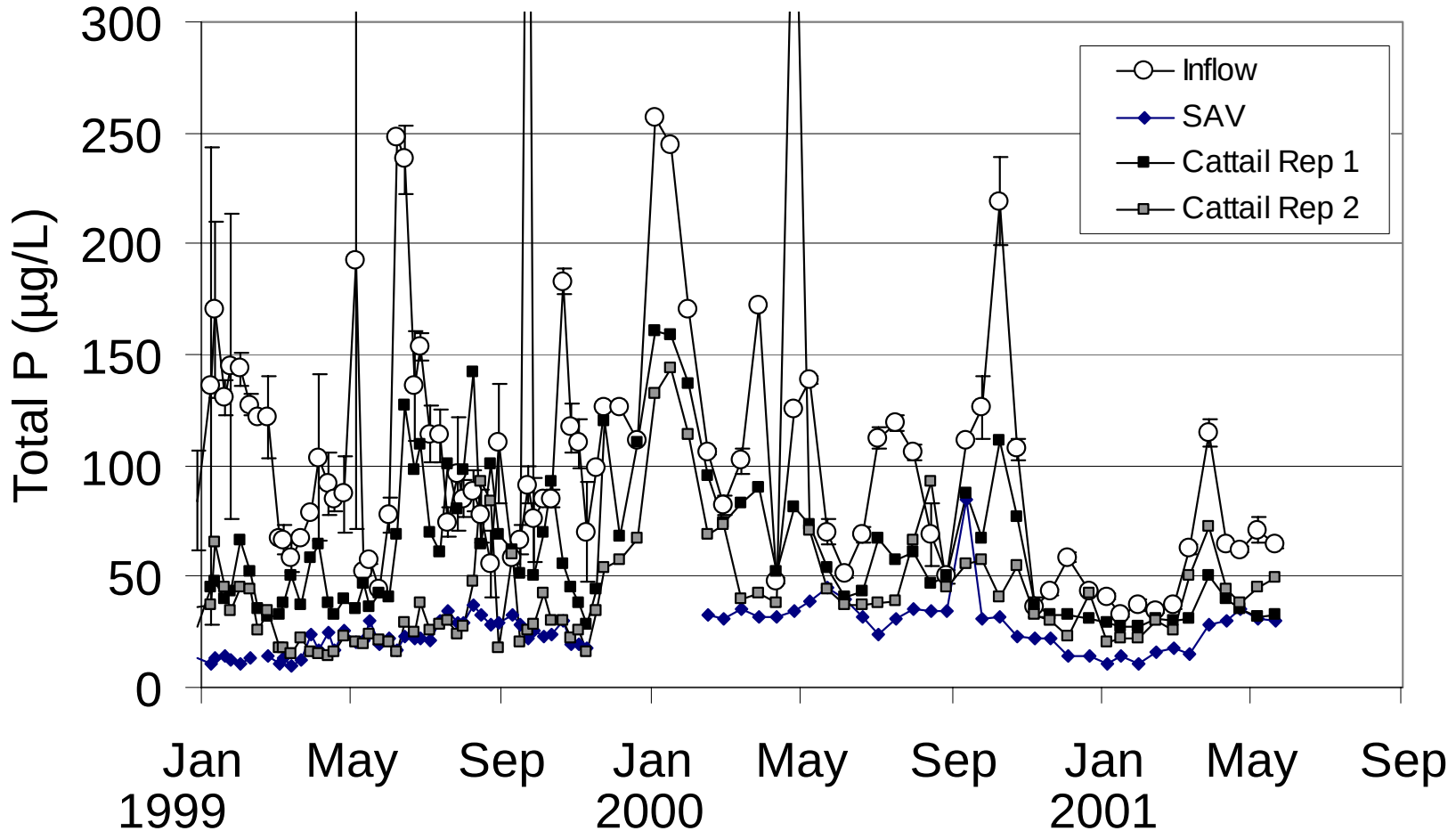

Figure 17. Total phosphorus concentrations in the inflow and outflow waters from one SAV-dominated and two cattail-dominated mesocosms that have received Post-BMP waters since December 1998. Inflow values represent means ± 1 s.d. of duplicate measurements.

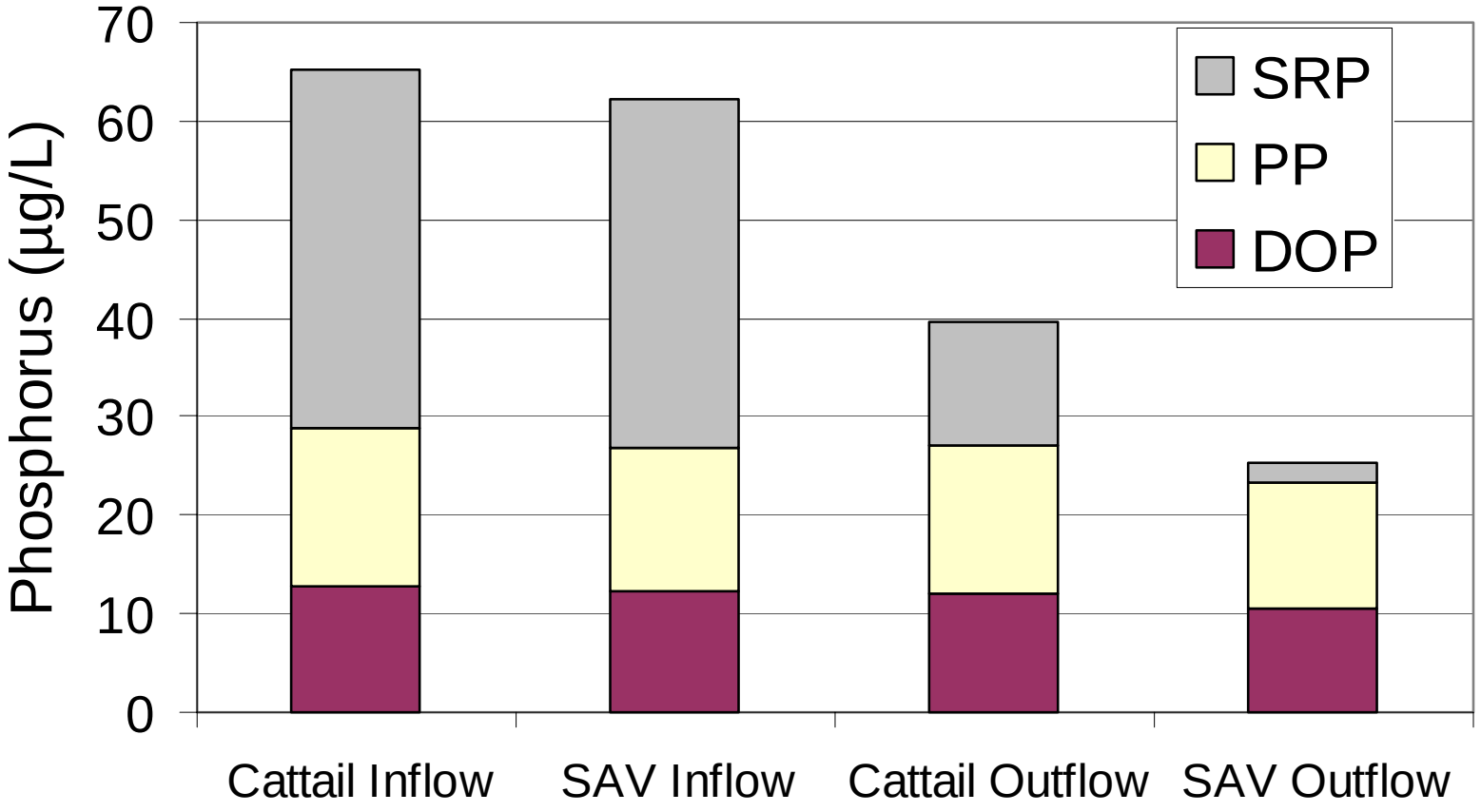

Figure 18. Mean dissolved organic, particulate, and soluble reactive phosphorus concentrations in the inflow and outflow waters from one SAV-dominated and two cattail-dominated mesocosms that have received Post-BMP waters since December 1998. Cattail outflow values represent means of duplicate mesocosms.

Effects of Flow Velocity on Phosphorus Removal by Shallow SAV/Periphyton Communities (Subtask 5viii)

Many investigators believe that flow velocity is an important variable in controlling the performance of SAV and periphyton systems. This parameter is difficult to test at the mesocosm scale, however, because any increase in flow (to increase velocity) also results in an increase in P loading. Also, to adequately address the effect of flow velocity on P removal using mesocosms, a long experimental platform is required.

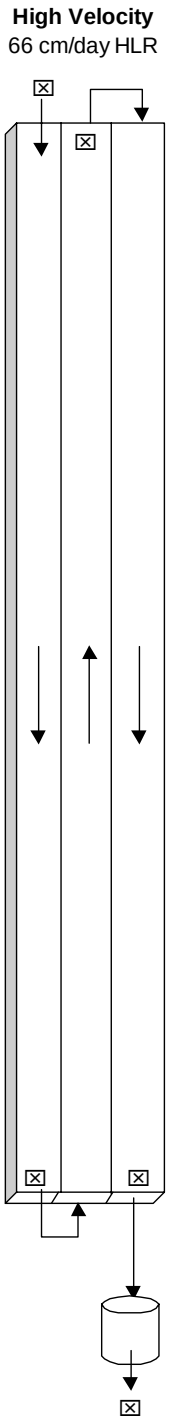

Figure 19. Modified flow path and sampling locations for the high velocity SAV/periphyton raceway experiment.

Upon completion of the shallow, low velocity periphyton/LR study (Subtask 5vii) on November 28, we joined the three 44 m long raceways together in series, thereby tripling the length of the flow path (Figure 19). We also tripled the inflow hydraulic loading rate to 66 cm/day, providing a velocity of 0.36 cm/sec. This flow rate is in the middle of the mean velocity range (0.2-0.5 cm/sec at average flow) proposed for the STAs. Inflow and outflow waters were analyzed weekly for TP for a six-month period under this modified operational regime.

During the beginning weeks of high velocity operation, *Chara* established dominance along the inflow region of the raceway, while calcareous periphyton dominated most of the remaining 132m long raceway. A mixed *Chara* /periphyton community dominated certain sections of the

raceway where the pre-existing community gradients had developed under the previous parallel flow paths of Subtask 5vii.

Over the six month study, the shallow raceway reduced TP concentrations in post-STA (STA-1W outflow) water from 23 to 17 µg/L, and the outflow limerock bed further reduced TP concentrations to 12 µg/L (Figure 20). This P removal performance is comparable to performance of the parallel raceways when flow velocities were lower (Table 9). Thus, to date the higher flow velocities do not appear to have a pronounced effect on P removal by this shallow system.

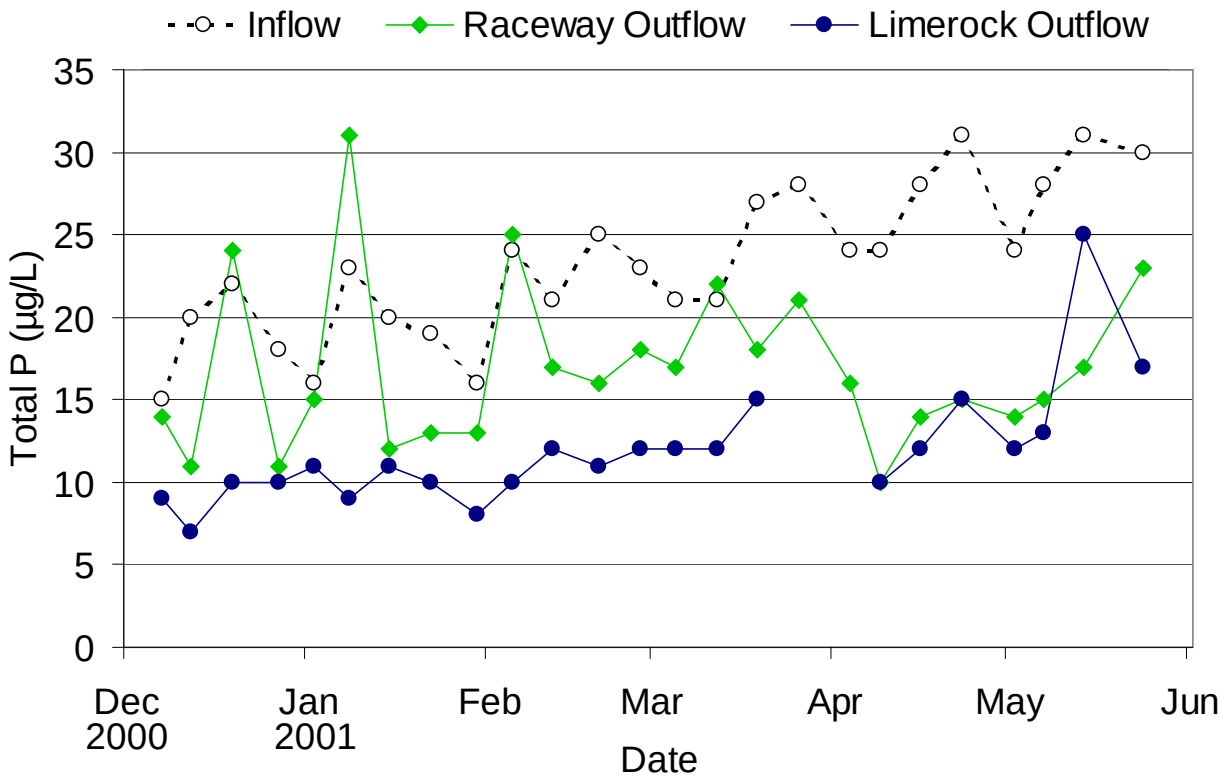

Figure 20. Total phosphorus concentrations in the inflow (Post-STA) waters and raceway and limerock outflow waters during the "high velocity" (0.27-0.36 cm/sec) operational period.

Table 9. Total P concentrations in the inflows and outflows from shallow SAV/periphyton raceways and outflows from subsequent limerock beds, during periods of high and low flow velocity. Removal efficiencies describe TP concentration reductions from inflow to limerock outflow.

	Replicate Raceways	Flow Velocity (cm/sec)	Inflow TP (µg/L)	Raceway Outflow (µg/L)	Limerock Outflow (µg/L)	Raceway/LR TP Removal (%)
July 2, 1998 - February 1, 2000	3	0.06	18	10	8	55
May 8, 2000 – November 28, 2000	2	0.12	23	15	11	52
December 7, 2000 – April 6, 2001	1	0.36	21	17	11	48
April 7, 2001 –May 24, 2001	1	0.27	28	15	15	46

Effects of Filter Media Size and Type on P Removal Performance (Subtask 5x)

Two media types – Pro-Sil™ Plus and limerock – were selected for further investigation based on the results from our original filter media experiment, concluded in November 2000. In that 13-week study, inflow TP concentrations were reduced from 17 to 14 and 13 µg/L, in small filter columns (5.8 L, HRT = 91–131 min.) containing limerock and Pro-Sil™ media, respectively. In order to more thoroughly understand the P removal potential of these filter media, we began the second round of filter media experiments at the SATT site during March. We wanted to evaluate the SRP, PP and DOP removal effectiveness of slightly larger filters, operated under steady-state hydraulic loadings.

Each of eight barrels (208 L) were filled with 30 cm of either limerock or Pro-Sil™ Plus (2.0-3.4 mm diam.) and plumbed to receive post-STA waters via gravity flow from a head tank (Figure 21). An external standpipe maintained a constant water depth at either 10 or 45 cm above the filter media. Outflow from the duplicate barrels containing limerock media at 45 cm water depth were fed in an upflow fashion into barrels containing 30 cm of limerock and 10 cm of overlying water. Outflow waters from each barrel (including each of the sequenced barrels), as

well as the Post-STA inflow, will be analyzed weekly for TP, SRP and pH during the 13-week monitoring period.

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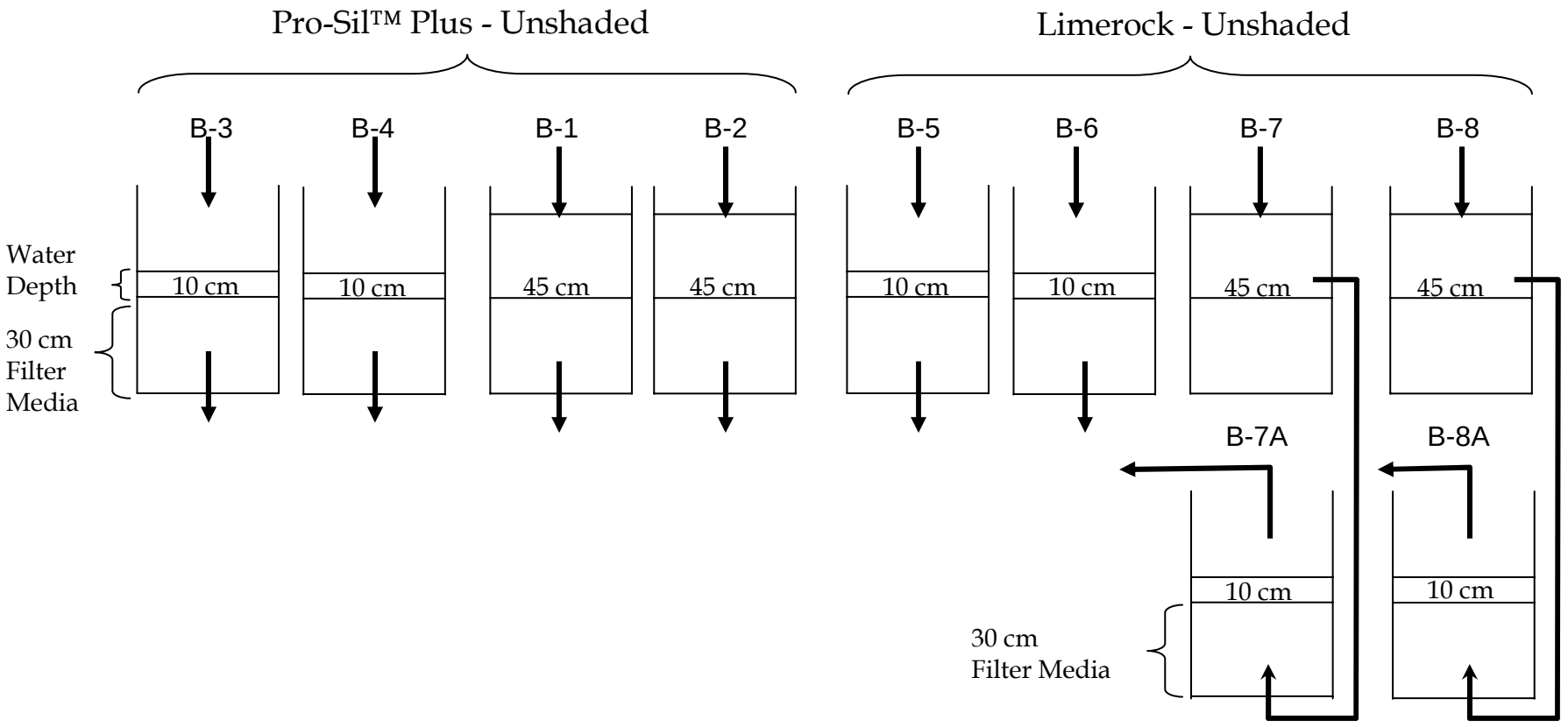

Figure 21. Schematic of the second filter media experiment operating at the SATT site with Post-STA waters.

Task 6. Test Cell Investigations

Operational Changes

We decided to increase the hydraulic (and therefore the P) loading rate to the north test cells (NTC-1 and NTC-15) during this 4-month reporting period in order to "challenge" the SAV community to achieve higher mass removals. The HLR to NTC-15 was increased from 5.1 to 11.7 cm/day on March 7, 2001, and then a further increase was initiated on June 1 when the HLRs of NTC-1 and NTC-15 were doubled from 12.1 and 11.7 cm/day to around 25 cm/day (Table 10). The HLR to the south test cells has been held constant at 5.5-5.7 cm/day since the beginning of September 2000.

As a result of a test cell water depth survey conducted by DBE personnel during this quarter, the water depths were adjusted by manipulating the weirs on April 6th to more desirable target depths (Table 10). These verified depths, along with the increase in HLRs in the two north test cells on June 1st, altered the HRT within each of the cells (Table 10).

Table 10. Previous and current water depths, hydraulic loading rates (HLR), and hydraulic retention times (HRT) in the test cells.

	Depth (m)		HLR (cm/day)		HRT (days)	
	Previous	**Current**	**Previous**	**Current**	**Previous**	**Current**
Test Cell	9/18/00 to 4/5/01	4/6/01 to present	9/18/00 to 6/1/01	6/1/01 to present	9/18/00 to 6/1/01	6/1/01 to present
NTC-1	0.74	0.60	12.1	26.8	6.1	2.2
NTC-15	0.96	0.60	8.1**	22.9	11.8**	2.6
STC-4	0.22	0.30	5.5	5.5	4.0	5.3
STC-9	0.45	0.45*	5.7	5.7	7.9	8.1

* Water depth was decreased to 0.30 m on April 6, 2001 and increased from 0.30 to 0.45 m on May 1, 2001
** Represents the time-weighted average of 5.1 cm/day from September 18, 2000 to February 23, 2001 and 11.7 cm/day from March 17 to June 1, 2001.

Vegetation Surveys

We have noticed visual changes in the SAV community throughout the test cell operational period, but had conducted only one quantitative survey prior to this quarter. We therefore conducted a qualitative vegetation survey of the four test cells on May 24, 2001.

At the inflow region of NTC-1, sparse duckweed (*Lemna*) and a dense stand of *Ceratophyllum* were present. *Najas* dominated the region between the inflow and the first quarter of the cell length, and was replaced with *Chara* for the rest of the length of the test cell. There were small patches of green filamentous periphyton present throughout. Overall, *Chara* dominated the cell and all plant populations appeared healthy.

Dense stands of *Ceratophyllum* were also present in the inflow region of NTC-15, along with some green periphyton. Thick beds of *Chara* persisted throughout the rest of the cell, except where it "thinned out" near the LR berm. Green filamentous periphyton inhabited both sides of the LR berm. There was one conspicuous triangular-shaped dead zone (10 ft x 10 ft x 20 ft) in the southeast corner of the cell. However, most of the cell was "topped out" by the SAV canopy.

Although very sparse, there was more emergent vegetation (*Scirpus*, *Typha*, *Panicum*) in the inflow region of STC-4 than in the other test cells. A healthy stand of "topped out" *Chara* dominated the SAV community throughout the cell. Grasses (e.g., torpedograss) occurred sparsely in the last quarter area of the cell.

Hydrilla colonized the inflow region of STC-9 in small patches. Green periphyton (filamentous algae) occupied the front quarter area where SAV was absent. Between one-quarter to one-half distance from the inflow, a dense stand of "topped out" *Chara* (with some *Najas*) dominated the remainder of the cell to the LR berm. Green filamentous periphyton existed on both sides of the LR berm.

Nitrogen Removal

Beginning October 4, 2000 and ending April 16, 2001, monthly grab-sampled inflow and outflow waters were analyzed for total Kjeldahl nitrogen (TKN), nitrite + nitrate (NO_x), and ammonium (NH_4). For the north test cells, the largest concentration reductions on a percentage basis were for NH_4-N (Table 11). Removal was higher in NTC-1 than NTC-15 for all the major nitrogen species. Even though there were higher nitrite + nitrate concentrations in the inflow of the south test cells than the north test cells, outflow concentrations at the south site were still below the detection limit. Total Kjeldahl N removal was inconsistent across the four test cells. Apparently organic nitrogen, the largest contributor to the TKN, is not amenable to removal in the test cells. The presence of a LR berm may have contributed some ammonium to the water column since the outflow concentrations of NH_4-N were higher in test cells with berms than in the comparable cells without berms.

Table 11. Mean monthly total Kjeldahl nitrogen (TKN), nitrite + nitrate nitrogen (NO_x-N), and ammonium nitrogen (NH_4-N) concentrations in the inflow and outflow of four test cells (NTC-1, NTC-15, STC-4, STC-9) dominated by submersed aquatic vegetation from October 14, 2000 to April 16, 2001. All values are in units of mg/L.

	NTC-1		NTC-15		STC-4		STC-9	
	Inflow	**Outflow**	**Inflow**	**Outflow**	**Inflow**	**Outflow**	**Inflow**	**Outflow**
TKN	2.58	2.12	2.50	2.49	2.48	2.67	3.86	2.21
NH_4-N	0.293	0.047	0.283	0.113	0.132	0.061	0.148	0.164
NO_x-N	0.030	0.022*	0.026	0.022*	0.136	0.022*	0.132	0.022*

* denotes concentrations below the detection limit.

Phosphorus Removal

Soluble reactive P continued to be the most readily sequestered P species for both sets of test cells (Figure 22 and Figure 23). Regardless of the inflow SRP concentrations, SRP has been nearly completely removed within all four test cells since weekly composite sampling was initiated in September of 2000.

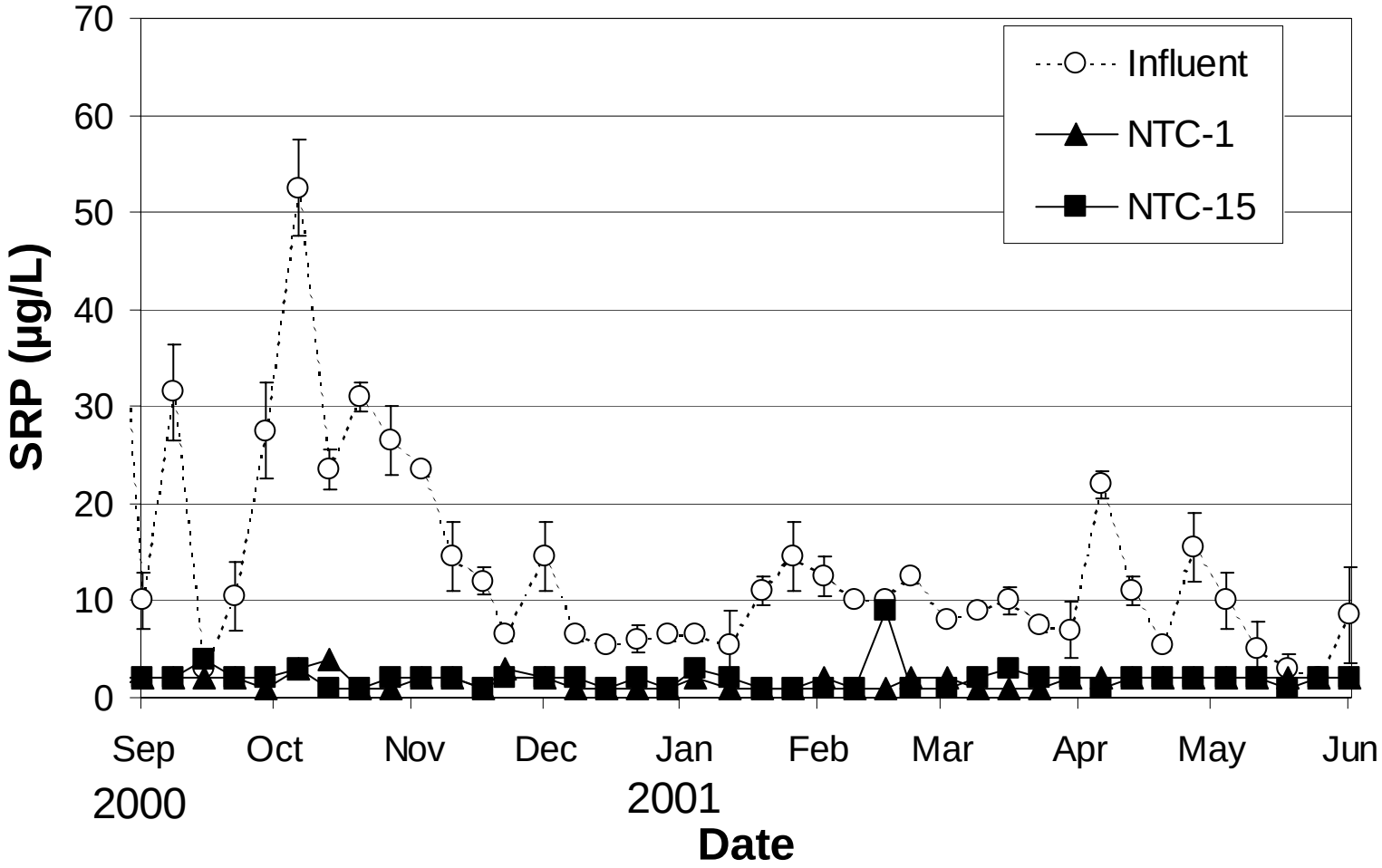

Figure 22. Soluble reactive phosphorus concentrations in the inflow and outflows from North Test Cells #1 and #15 from September 2000 through May 2001. Inflow values represent means ± 1 s.d. from duplicate measurements.

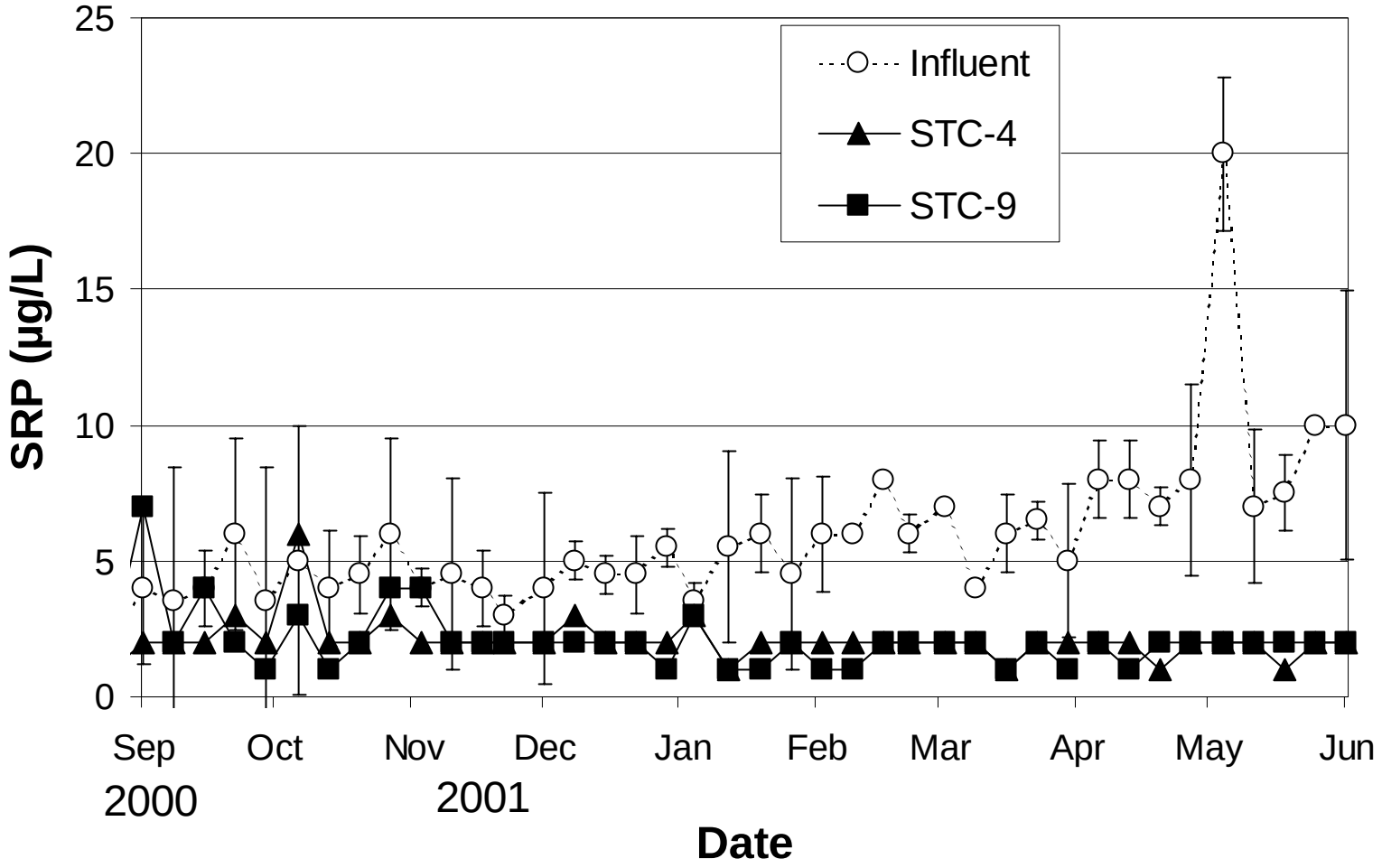

Figure 23. Soluble reactive phosphorus concentrations in the inflow and outflows from South Test Cells #4 and #9 from September 2000 through May 2001. Inflow values represent means ± 1 s.d. from duplicate measurements.

During the February - May reporting period, total P continued to be removed equally well in both NTC-1 and NTC-15 (Figure 24). The percentage decreases in P were 60 and 61% for NTC-1 and NTC-15, respectively. The presence of the LR berm at NTC-15 did not appear to enhance removal as the effluent total P concentrations for NTC-1 and NTC-15 each averaged 18 µg/L during the reporting period.

For the four-month reporting period, average outflow P concentrations were 22 and 21 for STC-4 and STC-9, respectively, which corresponded to removals of 12 and 8%. A continuous trend of net removal did emerge in May 2001 (Figure 25).

Chemical Treatment followed by Solids Separation

On March 5, 2001, District and HSA personnel began evaluating P removal performance of a Chemical Treatment/Solids Separation (CTSS) pilot treatment plant, operated in conjunction with an SAV-dominated wetland. Water from the North Head Cell is chemically treated by the pilot plant prior to discharge into NTC-14. Sample collection and analyses were provided by the District. Initial testing was conducted at a flow rate of 30 gpm (114 L/min), with additions of aluminum chloride (20 mg/L as aluminum) and an anionic polymer, Cytec Superfloc® A-130 Flocculant (0.5 mg/L). These conditions resulted in a CTSS effluent (prior to wetland discharge) TP concentration of 17 µg/L.

In order to achieve a system effluent TP concentration of ≤ 10 µg/L, the clarifier loading rate was decreased to approximately 20 gpm (76 L/min) and the coagulant and polymer dosing concentrations were increased to 40 mg/L as Al and 1.2 mg/L, respectively. These process modifications resulted in a CTSS effluent TP concentration of 8 µg/L. It was also noted that the pilot plant was not installed on a level platform, which has caused uneven loading to the clarifier. To compensate, District personnel installed V-notch weir plates onto the overflow trough surrounding the clarifier. The District also installed tube settlers in the CTSS effluent holding tank. This holding tank will allow settling of any floc discharged from the clarifier, and will result in further effluent clarification prior to discharge into NTC-14.

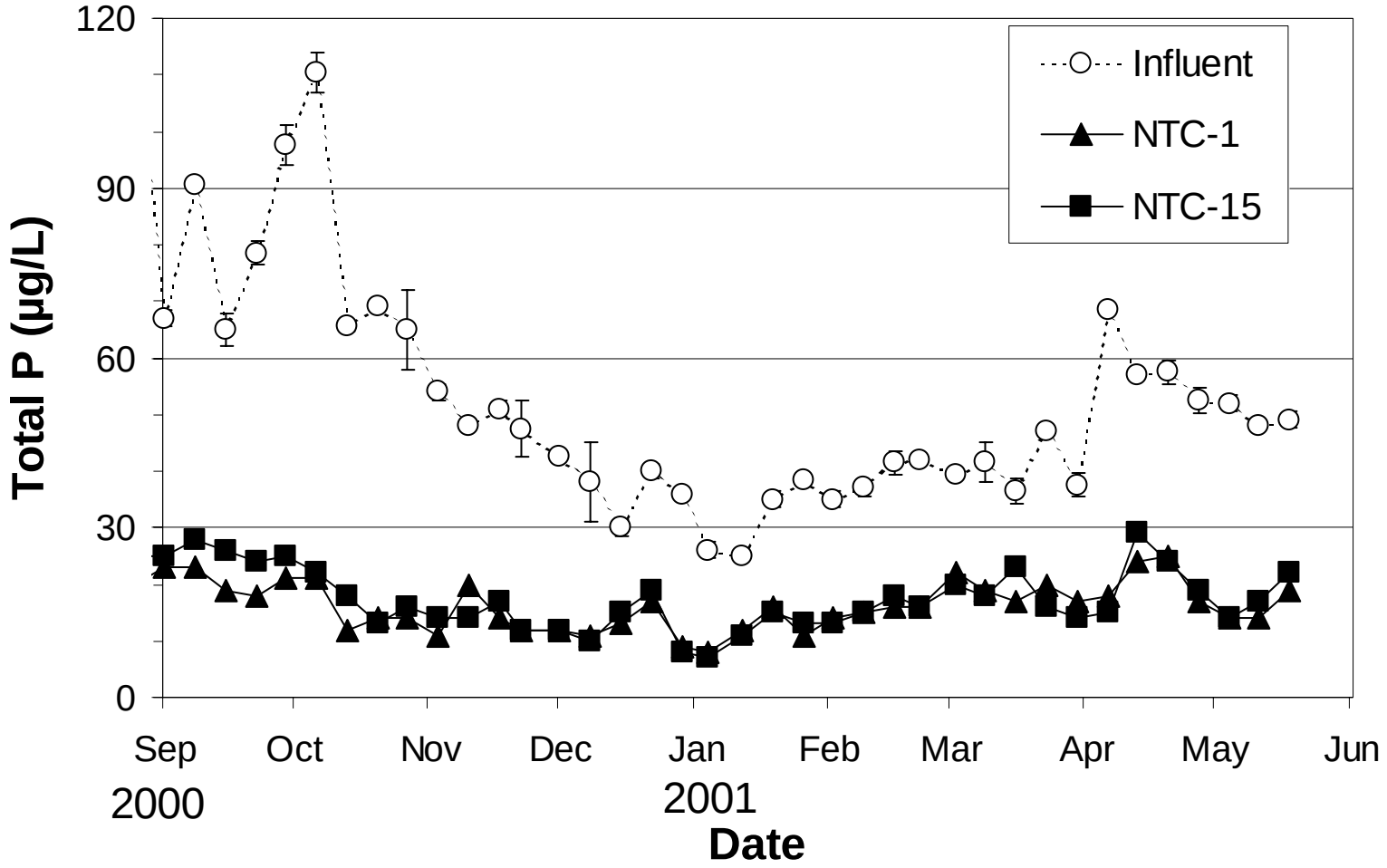

Figure 24. Total phosphorus concentrations in the inflow and outflows from North Test Cells #1 and #15 from September 2000 through May 2001. Inflow values represent means ± 1 s.d. from duplicate measurements.

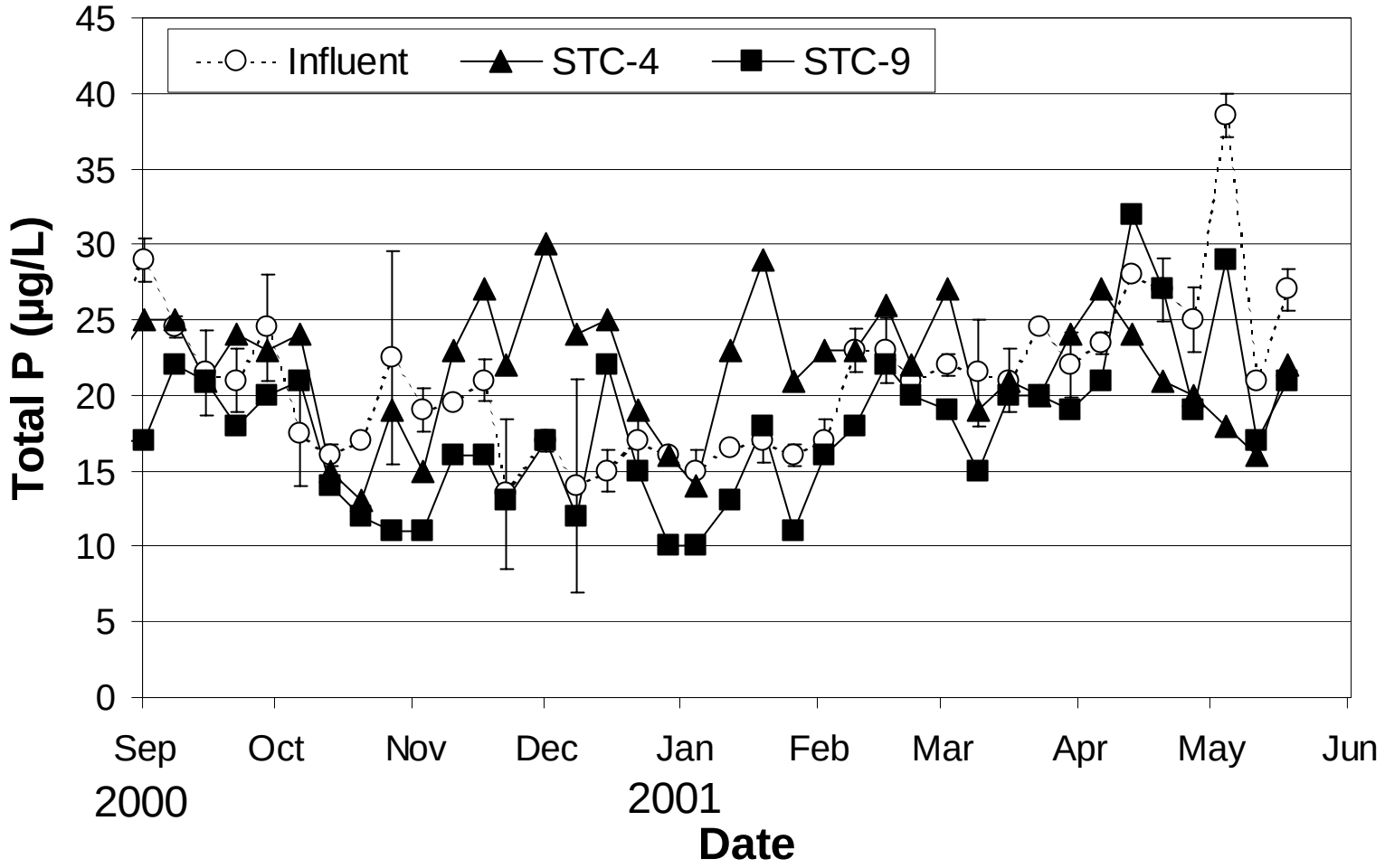

Figure 25. Total phosphorus concentrations in the inflow and outflows from South Test Cells #4 and #9 from September 2000 through May 2001. Inflow values represent means ± 1 s.d. from duplicate measurements.

Operation of the CTSS plant was suspended for equipment refurbishment on March 19, 2001, in preparation for 6-months of continuous operation. Beginning in April 2001, the pilot plant was operated at a flow rate of 15 gpm (57 L/min), which produced an average clarifier effluent TP concentration of 11 µg/L for April – May period of record. On May 1, 2001, clarifier effluent was discharged into NTC-14, an SAV-dominated cell, at a hydraulic loading rate of 0.33 cm/day. Weekly monitoring of effluent TP concentrations from NTC-14 began May 15. Preliminary data show slight increases (1-4 µg/L) in the TP concentration as water passes through NTC-14, but further sampling and analyses are still forthcoming. Operation of the pilot plant is scheduled to continue through August 31, 2001.

Task 9. Cell 4 Investigations

Performance Monitoring

On two occasions during the quarter (February 8, 2001 and April 12, 2001), and once during the prior quarter (December 19, 2000), we performed internal water quality sampling in Cell 4 along a 25-station grid (23 stations on December 19, 2000). All stations were located on a map of Cell 4 according to their GPS coordinates (Figure 26).

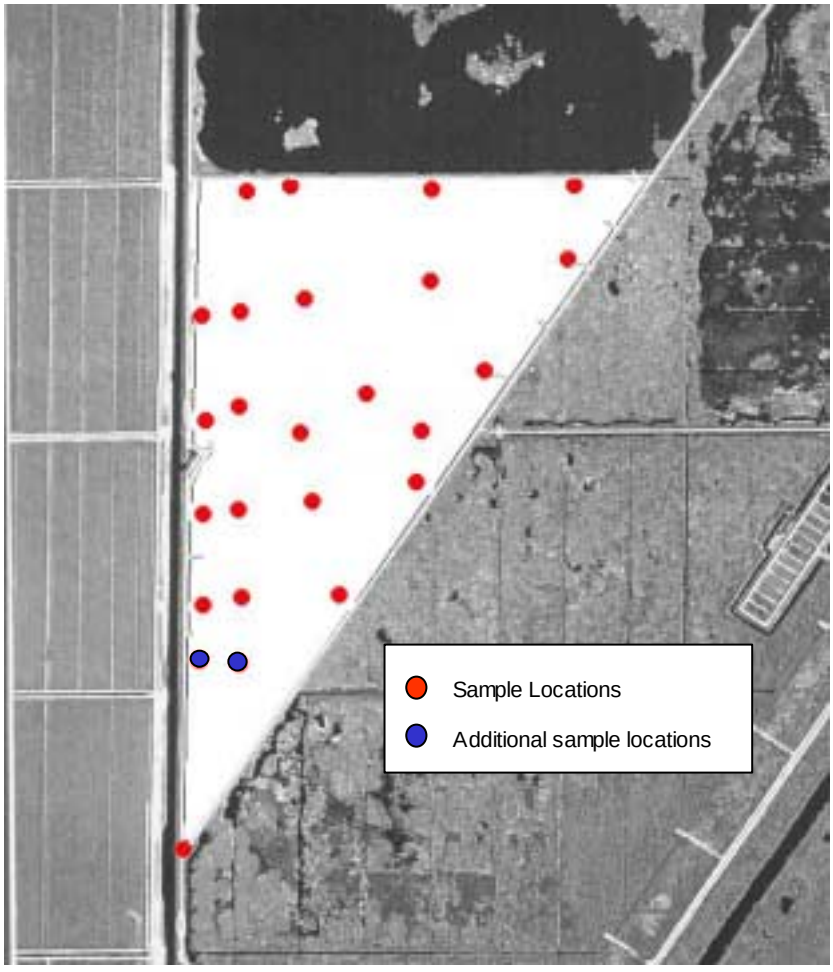

Figure 26. Sampling site location within Cell 4. The two additional sampling sites were added between the December 19, 2000 and February 8, 2001 sampling dates.

We used the computer program Arcview to generate 2-D spatial concentration gradients for Cell 4. These figures demonstrate large spatial and temporal variations in water column SRP and TP within Cell 4 (Figures 27 and 28). Concentrations of SRP were as high as 70 µg/L near the inflow culverts on December 19, 2000 and February 8, 2001, but were reduced to < 5 µg/L for nearly all of the cell on April 12, 2001 (Figure 28). The zones of higher SRP concentrations (along the western and northern levees) also exhibited higher TP concentrations (cf. Figures 27 and 28). The prominent and recurring longitudinal area of high P concentrations along the west levee corresponds to a path of short-circuiting that has developed since our first tracer study in late 1999. Our interpretation of the temporal variability in Cell 4 water column P levels will be facilitated when hydraulic loading rate data become available for each of the sampling days.

Stable Isotope Sampling and Preparation

Introduction

Most of the activity on this experiment reported for this quarter pertains to the development of a methodology for concentrating the dissolved carbon in the filtrates. We explored the efficacy of evaporating water samples at varying temperatures (40-100^{o}C) as an inexpensive means of concentrating filtrates. We also performed the first sample preparation for both particulate and dissolved carbon from the SAV-dominated north test cell 1 (NTC-1).

Methods Development

Particulate Residue

For the NTC-1 inflow and outflow samples, which were collected on January 26, 2001, 1-2 L of water were filtered through pre-combusted (500^{o}C for 2 hours) Whatman™ 0.45 µm pore size (47 mm diameter) glass fiber filters. A pre-rinse with about 50 ml of distilled water (which was discarded) preceded the sample filtration. After collection of the solid residue, several drops of 1 N HCl were added to each filter and fumed overnight. Filters were then washed with deionized water, and dried at 40-60^{o}C overnight before being shipped to the University of Alaska for stable isotope analysis (Table 12).

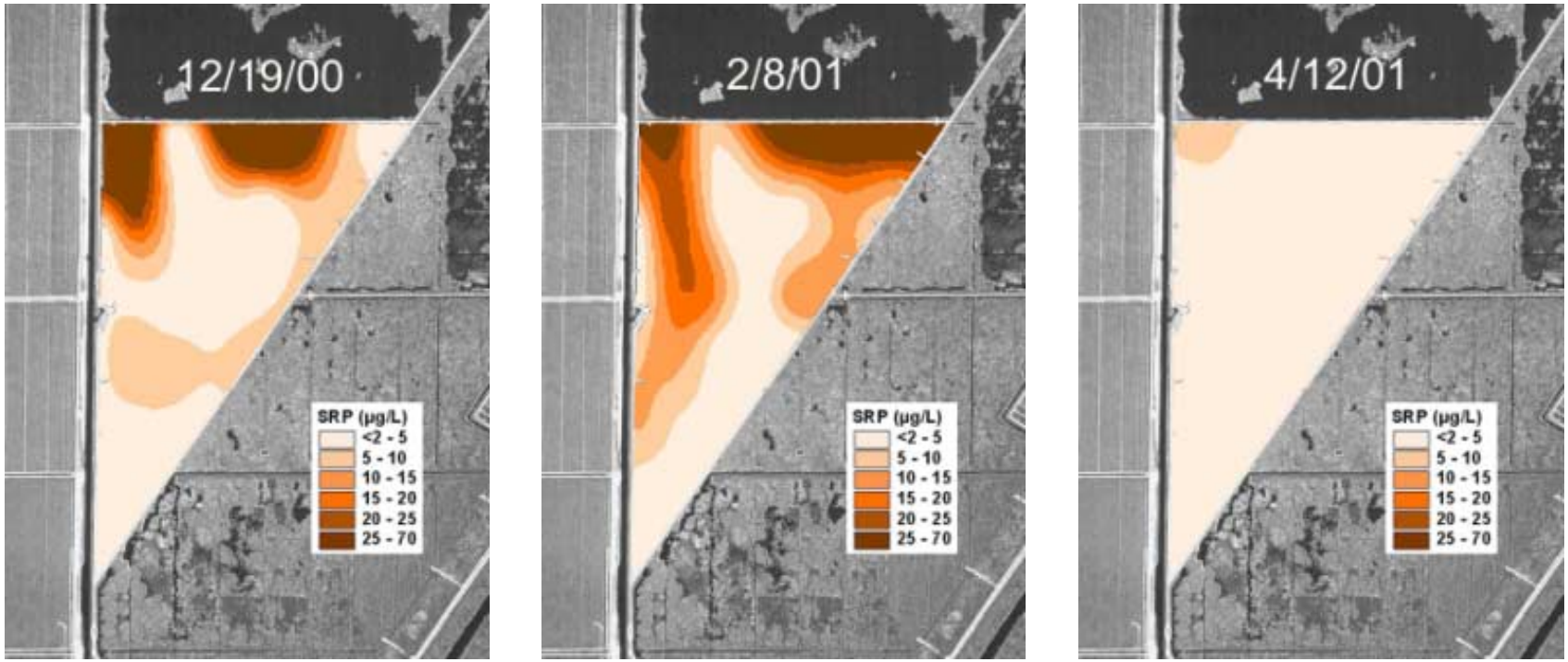

Figure 27. The distribution of soluble reactive phosphorus concentrations (µg/L) within Cell 4 on three separate dates (Dec. 19, 2000; Feb 8, 2001; and April 12,2001).

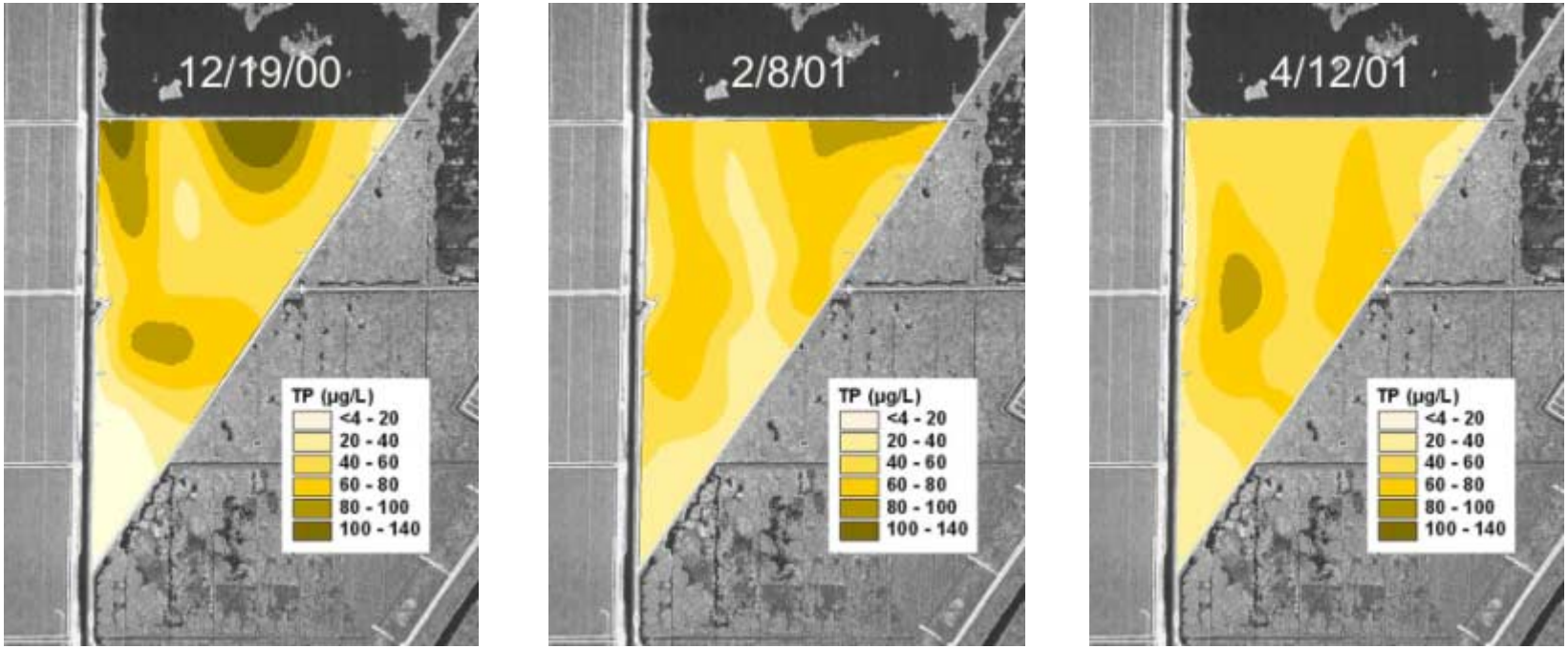

Figure 28. The distribution of total phosphorus concentrations (µg/L) within Cell 4 on three separate dates (Dec. 19, 2000; Feb 8, 2001; and April 12, 2001).

Table 12. Stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) in particulate organic matter filtered from surface waters collected in the inflow and outflow of NTC-1 on January 26, 2001.

Sample	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Ave. $\delta^{13}C$ (‰)
Dist. H_2O Blank			
NTC-1 In-A	-2.07	-33.9	-34.4
NTC-1 In-B	0.56	-35.0	
NTC-1 Out-A	3.15	-29.8	-30.4
NTC-1 Out-B	-2.43	-31.0	

Dissolved Residue (Filtrate)

After filtering the particulates, the filtrates were frozen until February 26, at which time they were thawed, subdivided into three aliquots of 600-mL, and placed into 1-L Pyrex beakers. On the following day, each aliquot was placed into a drying oven at one of three temperatures: 60, 80 or 100°C, and allowed to evaporate to dryness. A 30 mg/L peptone standard was also dried at each of the three temperatures. After 3 days, the 600-ml water sample was completely evaporated under each of the 60, 80 and 100°C drying temperatures. Whereas the dried residue of the sample waters was easily scraped from the sides and bottom of each beaker, the residue from the dissolved 30 mg/L peptone could not be scraped and isolated because of the firm adherence of the thin coat of peptone to the glass surfaces.

Although the results of this initial test were encouraging for both particulate and dissolved residues, in that it appeared that temperature did not affect the carbon isotope ratio (Table 13), the exclusion of a peptone standard (because of failure to recover the dried 30 mg/L peptone standard) meant that we could not state with confidence that temperature effects were unimportant in determining the carbon isotope ratio. We therefore initiated a second temperature-effect study by increasing the concentration of the peptone standard from 30 to 300 mg/L, and then exposing the higher concentration to temperatures of 40, 60 and 80°C. We found that it was easier to recover the dried peptone residue at this higher concentration, especially at 40°C. The results of the isotope and elemental concentration analyses were encouraging (Table 13). Despite losses in the N and C concentrations that increased with temperature, the $\delta^{13}C$ was unaffected. This means that although temperature affects the carbon

and nitrogen contents of the peptone, the C-13 and C-14 components of the dissolved organic matter are equally affected, resulting in the ratio remaining unaltered.

Standard Preparation Procedure for the Dissolved Residue (Filtrate)

As a result of these preliminary trials, the following procedure will be followed when concentrating the filtrates from sampled stations within the north test cells and STA-1W. If the filtrate cannot be concentrated immediately, it will be kept frozen during the interim. The concentration process will consist of evaporating each sample filtrate to dryness at 40^{o}C. Our experience indicates that it takes 4-5 days to evaporate a 1L sample to dryness at this temperature. After reaching dryness, the dried filtrate residue will be scraped from the sides and bottom of the beaker. After an overnight fumigation with 1 N HCl, the residue is rinsed with distilled water and re-dried before being shipped to the University of Alaska for isotope analysis. An internal sample (peptone Hy-Soy J enzymatic hydrolysate, Sigma Lot # 46H0862) will also be dried from solution and recovered in the same manner as the sample water residue. All samples, blanks, and internal standards for both particulate and dissolved residues will be processed in duplicate.

Table 13. Effects of different evaporation temperatures on the recoveries of N, C, δ ^{15}N, δ ^{13}C in peptone and dissolved organic matter from surface waters collected in the inflow and outflow of NTC-1 on January 26, 2001.

Sample	Temp (°C)	% N	% C	δ ^{15}N (‰)	δ ^{13}C (‰)	Ave. δ ^{13}C (‰)
Peptone-A	Undiss	8.26	39.1	0.76	-24.7	-24.8
Peptone-B	Undiss	8.43	38.7	0.59	-24.9	
Peptone-A	40	7.04	32.4	1.78	-24.4	-24.3
Peptone-B	40	7.37	34.1	1.85	-24.2	
Peptone-A	60	6.77	32.4	3.35	-24.3	-24.5
Peptone-B	60	6.96	34.8	2.99	-24.7	
Peptone-A	80	5.81	30.8	1.75	-24.2	-24.4
Peptone-B	80	5.62	30.6	1.62	-24.7	
NTC-1 In-A	60	0.21	5.0	3.92	-20.4	-20.5
NTC-1 In-B	60	0.21	4.9	3.79	-20.6	
NTC-1 In-A	80	0.22	4.9	3.65	-21.3	-21.5
NTC-1 In-B	80	0.23	5.0	3.58	-21.7	
NTC-1 In-A	100	0.19	4.6	4.05	-20.4	-20.7
NTC-1 In-B	100	0.20	4.7	3.63	-21.0	
NTC-1 Out-A	60	0.17	3.2	3.94	-24.6	-24.7
NTC-1 Out-B	60	0.21	3.9	3.98	-24.7	
NTC-1 Out-A	80	0.20	4.1	3.90	-22.9	-22.9
NTC-1 Out-B	80	0.23	4.7	3.54	-22.8	
NTC-1 Out-A	100	0.20	4.1	3.50	-23.0	-23.0
NTC-1 Out-B	100	0.20	4.0	3.61	-22.9	

Task 10. Cell 5 SAV Inoculation and Monitoring

On February 14, 2001, we performed our fifth quarterly intensive sampling effort to characterize the SAV populations of Cell 5. *Najas guadalupensis* and *Ceratophyllum demersum* were still found to be the most prevalent species throughout the cell, but their densities had decreased in February compared to November 2000 (Figures 29 and 30). On the other hand, *Hydrilla* sp. increased in biomass during the same 5-month period (Figure 31). The reduction in the *Najas* and *Ceratophyllum* biomass may have been due to increased competition by *Hydrilla*, or to winter-time seasonal factors. Our next, and final, survey this summer will provide a more definitive picture of whether *Najas* and *Ceratophyllum* populations are being reduced because of competitive exclusion from *Hydrilla* sp., or whether the observed reduction was due to seasonal factors.

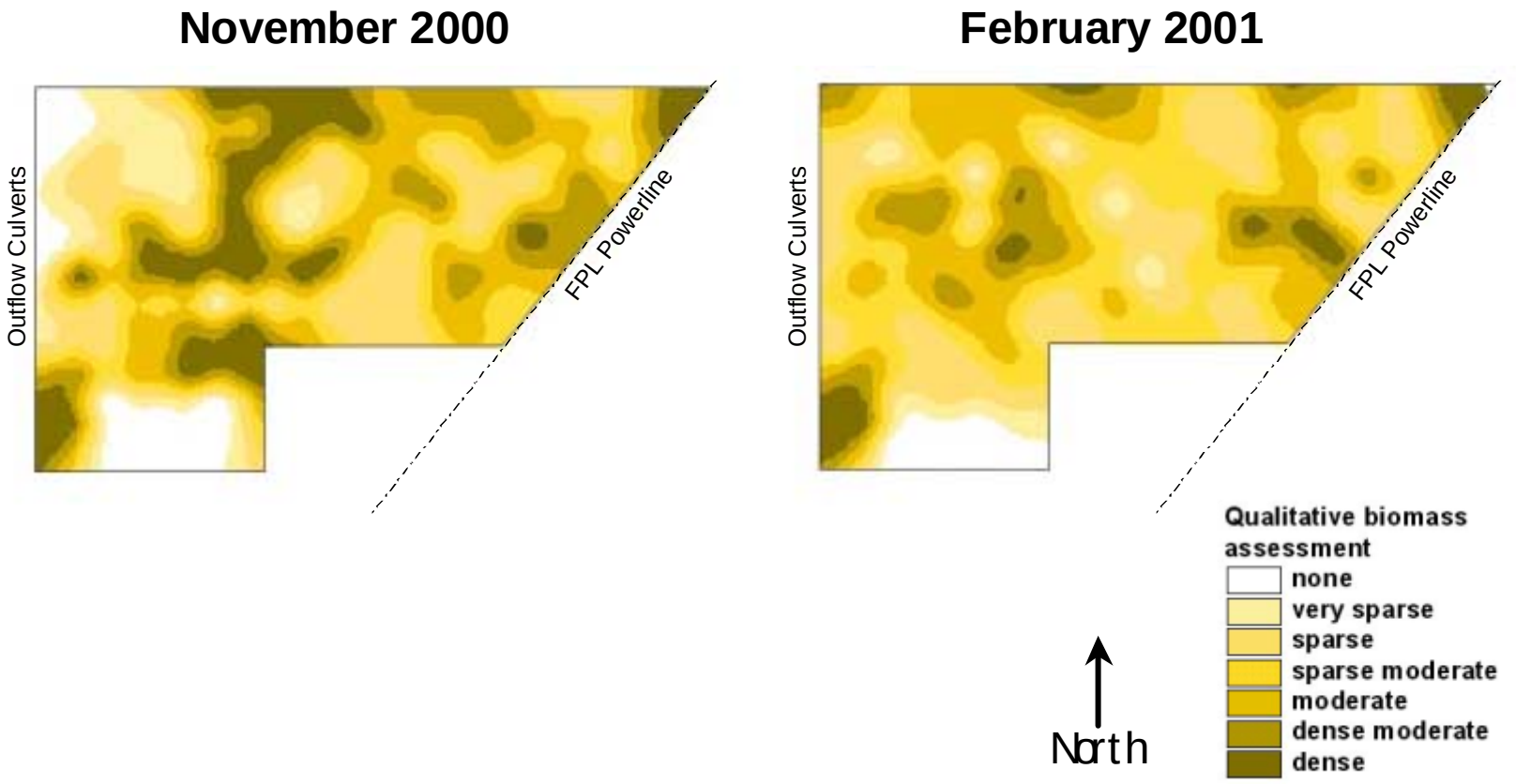

Figure 29. Cell 5 SAV Colonization: Presence and distribution of *Najas* during two 120-station visual surveys.

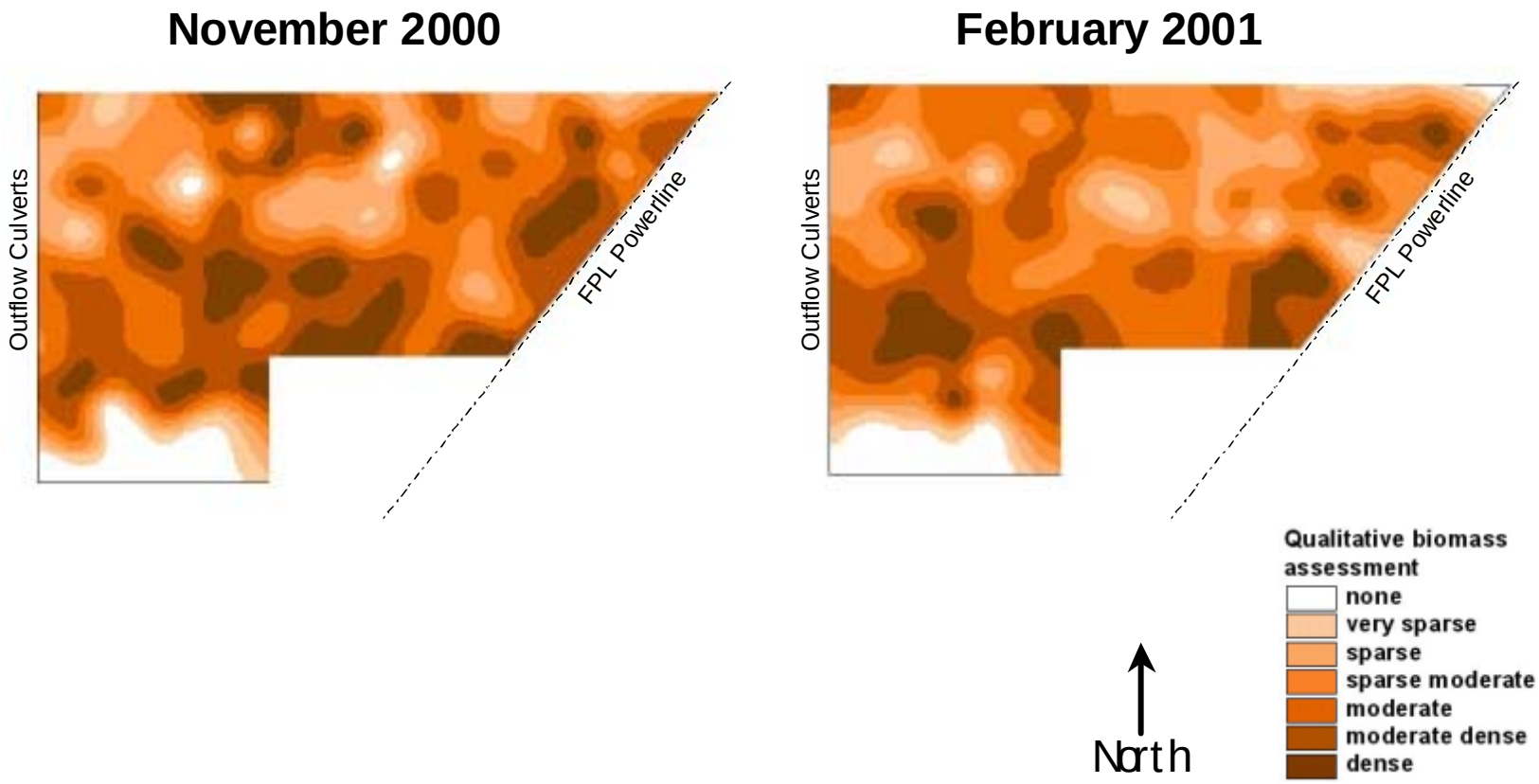

Figure 30. Cell 5 SAV Colonization: Presence and distribution of *Ceratophyllum* during two 120-station visual surveys.

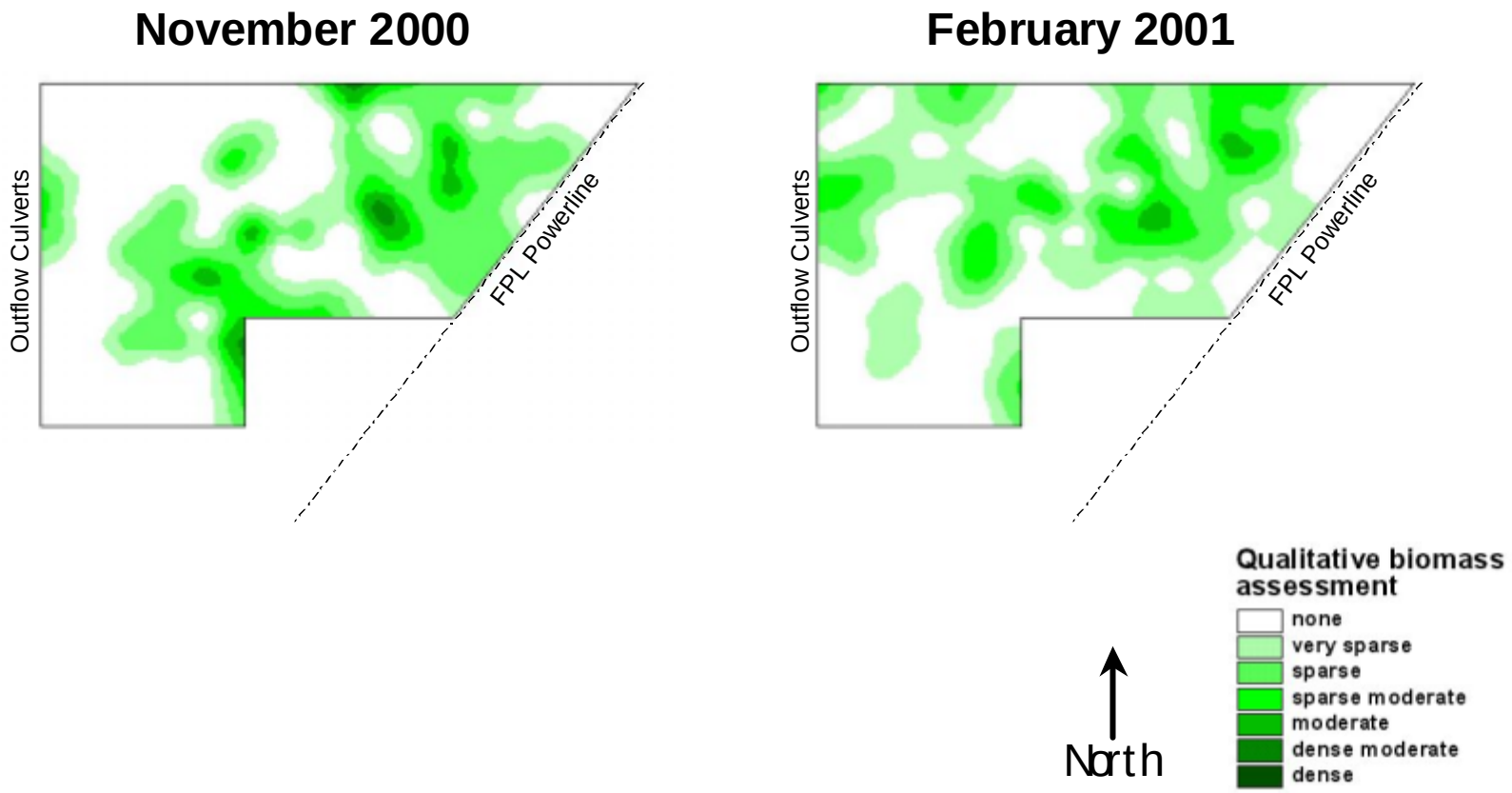

Figure 31. Cell 5 SAV Colonization: Presence and distribution of *Hydrilla* during two 120-station visual surveys.